Title: A multibiomarker study to delineate the effects of environmental stressors on aquatic shellfishes of West Bengal

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Registration Number with date:

R-18RS218210201 dated 11th December, 2020

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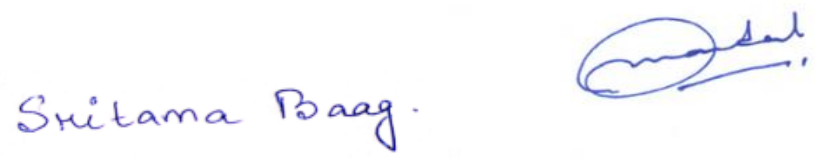

Signature of Research Scholar **Signature of Supervisor**

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I. Abstract

Industrial development and human population expansion are identified as the major causes behind climate change globally. Environmental disturbance as a consequence of global climate change imposes deleterious threats to marine ecosystems. The purpose of the present doctoral thesis work is to delineate the physiological energetics and oxidative stress in two economically important shellfishes from West Bengal, *Bellamya bengalensis* (freshwater species) and *Scylla serrata* (estuarine species) after exposure to different environmental and anthropogenic stressors. The aim of the work is to study the response of shellfishes habitated in both freshwater and marine realm as these ecosystems are diverse in nature. Significant contrasting changes were observed in physiological energetics and oxidative stress in both the species. The results obtained clearly highlights the detrimental combined impact of global climatic factors and pollution on the shellfishes that might potentially reduce their population and affect regional aquaculture in forthcoming years.

II. Introduction

The aquatic ecosystems are encountering numerous environmental challenges and myriad anthropogenic perturbations from the past few decades. Extortionate volume of atmospheric CO_2 release is majorly held responsible for global climatic alterations. Observing current trends, an increase of 2–4°C average surface water temperature in conjunction with reduction of pH by up to 0.4 units is anticipated by the year 2100 due to the incessant increase of atmospheric greenhouse gas concentration (IPCC, 2014, 2019). This constant decrease in pH and temperature elevation has been occurring from the past few decades and are named as ocean acidification and ocean warming respectively. Due to this drastic change in climatic conditions owing to anthropogenic perturbations our present geological time is recognized as the Anthropocene (“the human era”). Global environmental drivers affect an organism’s sensitivity to pollution and might also modify their toxicity potential by altering the bioavailability. Freshwater ecosystems are under continuous anthropogenic pressure due to their proximity to human settlements (Davies and Vethaak, 2012). Widespread use of pesticides in modern agricultural practice has a significant contribution in contaminating freshwater bodies across the world and has become a potential threat to mankind (Zhang and Zhang, 2011; Mondal et al., 2018). Following industrial revolution, global trade, mainly petrochemical transportation has rapidly intensified. Oil spill is one of the most pervasive anthropogenic menaces on coastal ecosystem (Hawkins et al., 2017; Uno et al., 2017;

Lumibao et al., 2018). Pollutants can hinder the capacity of animals to continue homeostasis in an altering climate, which might in turn reduce population perseverance and disturb ecosystem functioning (Baag and Mandal, 2022).

It is now unequivocally acknowledged that predicted climatic changes will execute significant costs on physiological functioning of most species. Ectotherms are of specific concern as their physiological functioning mostly relies on the external environmental drivers (Dissanayake, 2014). These species are more susceptible as they lack internal buffering mechanisms with varying external environment (Nilsson and Lefevre, 2016). Tropical invertebrates are more vulnerable as happen to be stenothermic with constricted thermal tolerance range and inhabit areas of their optimal thermal boundary (Deutsch et al. 2008).

Aquatic organisms undergoing rigorous environmental oscillations have diverse responses and adjustment capacity (Madeira et al. 2018). Compromising the energy budget is an essential mechanism for survival under stressful situations for aquatic ectotherms (Baag et al. 2020). In recent times, an efficacious physiological index namely scope for growth (SfG) has gained attention which calculates the energy allocation in a particular animal (Bayne and Newell 1983; Baag et al. 2020). It evaluates the excess energy left for growth and reproduction after all the essential metabolic activities are completed (Genoni and Pahi-Wohstl 1991). Higher metabolic energy demand as a result of increasing temperatures leads to a deficit in dissolved oxygen content of the water bodies, constraining animals' aerobic scope (Deutsch et al. 2015). Thermal performance curves are used to apprehend an organism's response to stress by enumerating the effect of body temperature on an ectotherm's performance and fitness level (Sinclair et al. 2016).

Temperature escalation also incites surplus production and accretion of reactive oxygen species (ROS) that lead to oxidative damage in various cellular components (Lesser, 2006; González et al., 2010; Zhou et al., 2010). Basal amount of ROS is produced as a result of regular metabolic/physiological functioning like cell signalling and homeostasis. Living organisms possess a highly developed antioxidant defence system to eradicate and balance additional ROS (Lushchak and Bagnyukova, 2006; Sookruksawong et al., 2013). The spontaneous balance between production and removal ROS is essential to maintain an animal's health. The antioxidant defence system includes some antioxidant and detoxifying enzymes, primarily superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) obligatory for ROS elimination. If ROS quenching is not sufficient, lipid peroxidation

(LPO) level rises in cell (Regoli and Giuliani, 2014). Surplus ROS production debilitates the antioxidant defence machinery and cause damages to cellular DNA (Bhagat et al., 2017). Biomarkers can be utilized to sense ROS and understand the production-elimination equilibrium, though results from specific enzymatic and non-enzymatic biomarkers are often complicated. Multiple-biomarker methods are more suitable (Beliaeff and Burgeot, 2002), with scores of biomarkers from numerous levels of biological organization leading to an enhanced idea of stress response mechanisms. However, due to the inconsistency in internal functioning involved within the organism in presence of multiple factors, a collective biomarker response from different biological levels can offer a holistic picture as a single numeric value (IBR) in bio monitoring studies.

Economically important shellfishes like molluscs and crustaceans are key members of ecologically sustainable aquaculture which can also serve as excellent candidates for bio-monitoring programs. *Bellamya bengalensis* is the most abundant freshwater snail in India and is of great economic importance as it serves as a food item for some aquatic animals as well as a delicacy for human beings (Datta et al., 2016). The mud crab (*Scylla serrata*) is one of the most commercially prominent species for aquaculture in coastal parts of India (Paital and Chainy, 2014).

Despite substantial research endeavour on aquatic invertebrates, the biological ramifications of various global and local environmental factors have gained attention independently however; research works on their combined effect are still very limited. Albeit its importance, inadequate information exists on the ecophysiology of *B. bengalensis* and *S. serrata*. Thus, it is requisite to study the physiological functioning of these species under global change scenarios and pollution, as they hold a vital position in the aquaculture and rural economy of the country.

III. Objectives

Objective 1: Effect of environmental stressors on physiological energetics and energy budget of experimental animals.

Objective 2: Effect of environmental stressors on thermal adaptation pattern and estimate the thermal tolerance levels of experimental animals.

Objective 3: Effect of environmental stressors on some enzymatic and non-enzymatic oxidative stress biomarkers of experimental animals.

Experiment 1: (Objective 1&2) (Baag et al., 2020; Journal of Thermal Biology)

IV. Methodology

The purpose of the study was to interpret the physiological response and alterations in energy allocation mechanism of *Bellamya bengalensis*, in the projected elevated temperature conditions in the imminent times by evaluating the growth, body conditions, physiological energetics (ingestion rate, absorption rate, respiration rate, excretion rate and Scope for Growth) and thermal performance, Arrhenius breakpoint temperature (ABT), thermal critical maxima (CTmax), warming tolerance (WT) as well as thermal safety margin (TSM) through mesocosm experiment. Adult *Bellamya bengalensis* (26±2mm) were collected from a well-managed pollution free freshwater pond in Budge Budge, South 24-Parganas, West Bengal, India. The animals were exposed to three different temperatures, 25°C (average habitat temperature the animal resides) and higher temperatures 30°C, 35°C for one month (30 days). Individuals were kept in covered plastic aquaria (40 X 28 X 20cm) in three replicates (each holding five animals of 26±2mm) per treatment. Food (Fresh Iceberg Lettuce) was provided every day. Variations in energy budget were estimated on 15^{th} and 30^{th} day. Thermal performance test was conducted on 32^{nd} day of the experiment.

Ingestion rate (I = dry weight of total food ingested/hour) and assimilation efficiency (AE (%) = [F'-E'/(1-E') (F')] *100) where F' and E' denotes organic portion of food and faecal matter respectively (Conover, 1966) were calculated. Absorption rate (Ab) was measured by I*AE. Respiration rate (R) was estimated by determining the lowering of dissolved oxygen concentration in a locked chamber and excretion rate (E) was measured following phenol-hypochlorite method (Solorzano, 1969). All the physiological parameters were transformed into its energy equivalent (Elliott and Davison, 1975) to finally estimate the energy budget or Scope for Growth {SfG = Ab – (R+E)} following Widdows (1985). An increasing thermal ramp ($2^{o}C\ h^{-1}$) was used to evaluate the thermal fitness in shellfishes by determining the respiration rates at every ramping temperature for all the treatment groups to understand the thermal acclimation pattern after the 3 temperature treatments following Giomi et al. (2016). The critical thermal tolerance level was determined based on the fluctuating respiration rates as well as their locomotory behaviour throughout the thermal ramping procedure (Lutterschmidt and Hutchison, 1997).

Statistical analysis:

To investigate the effect of temperature and duration of stress (number of days) on total weight, body conditions, ingestion rate, assimilation efficiency, absorption rate, respiration rate, excretion rate, scope for growth and the thermal performance a two-way per mutational analysis of variance (PERMANOVA) was applied. The main test was done using temperature and duration as fixed factors and whenever significant interaction were found between these factors, a paired test was performed for each factor separately in each level from other factor for the energetics studies. For thermal performance analysis the factors taken into account were acclimation temperatures and ramping temperatures. All the analyses were performed in PRIMER 6 with PERMANOVA+ add on.

V. Results

Substantial changes were detected in body conditions and physiological energetics of snails. A significant decline in total weight was observed on both 15^{th} and 30^{th} days for 30ºC and 35ºC treatment groups compared to 25ºC (Fig. 1a). A statistically significant variation was also noted in organ to flesh weight ratio (Fig. 1b) amongst the temperature treatments and the value decreased with rising temperature with least at 35ºC and maximum at 25ºC. Both ingestion and absorption rates varied significantly on 15^{th} and 30^{th} day with the value in 25ºC being greater than that of 30ºC and 35ºC treatments (Fig. 2a&b). On 15^{th} and 30^{th} days significant alterations were observed among all the temperature treatments with respiration rate found lowest in 25ºC and maximum in 35ºC group (Fig. 2c). On 15^{th} day a significant increase in excretion rate for 30ºC groups was noted than 25ºC groups while on 30^{th} day there was a significant rise in excretion rate for 35ºC treatment than the other groups (Fig. 2d). On both 15^{th} and 30^{th} day noticeable changes were found amid all the temperature groups with SfG being least at 35ºC group and maximum at 25ºC group (Fig. 3). Although no considerable impact on ABT/CTmax was observed, the peak of thermal performance curve significantly lowered for animals that were reared in higher temperature treatments (Fig. 4). Based on locomotory changes, CTmax was observed to be at 36ºC for this species. The warming tolerance level and thermal safety margins were estimated 11ºC and 9ºC respectively.

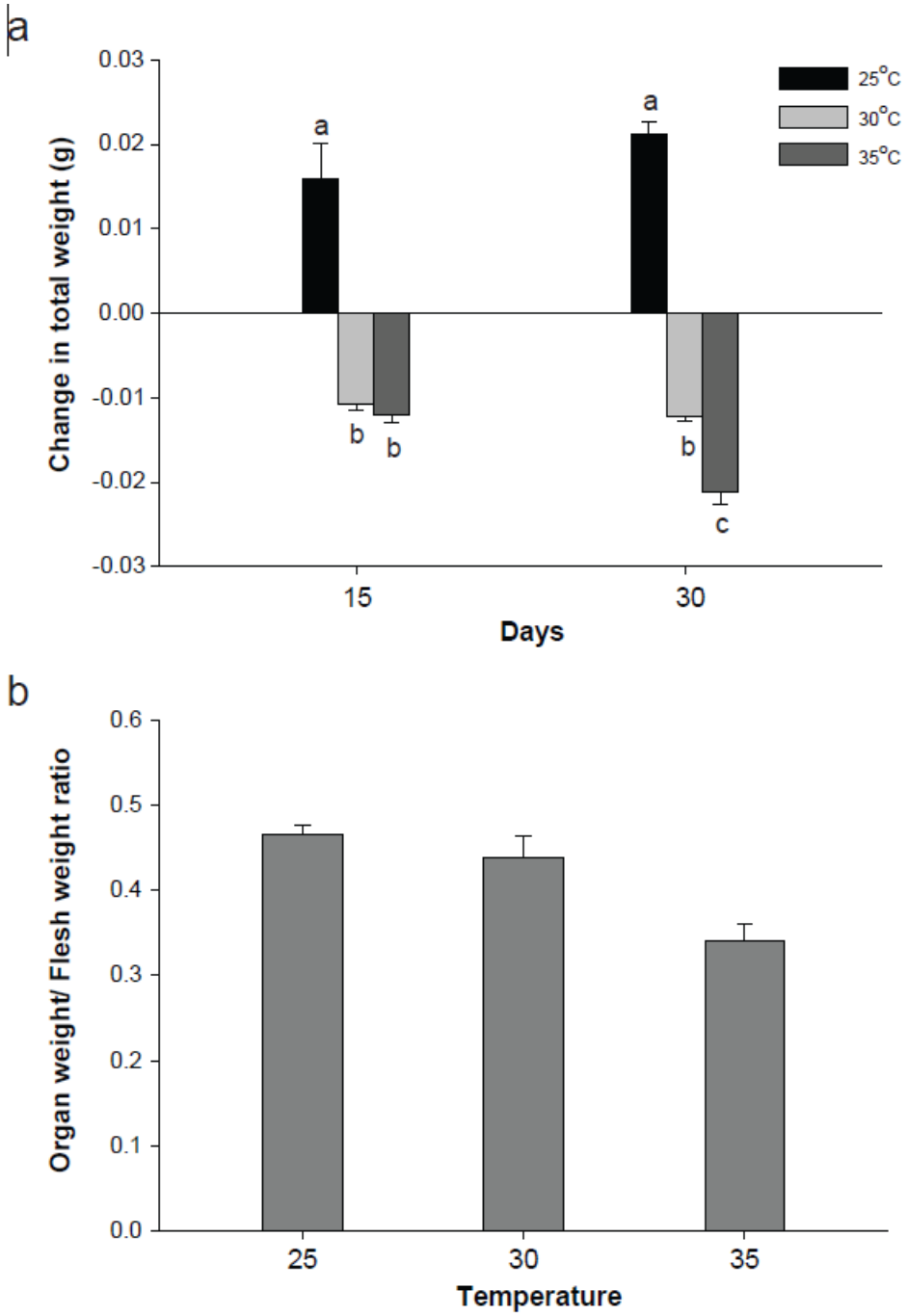


Fig.1. Change in total weight (a) and change in organ to flesh weight ratio (b) of *Bellamya bengalensis* under different temperature treatments (25ºC, 30ºC and 35ºC) during the experimental period. Values represent mean ± SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

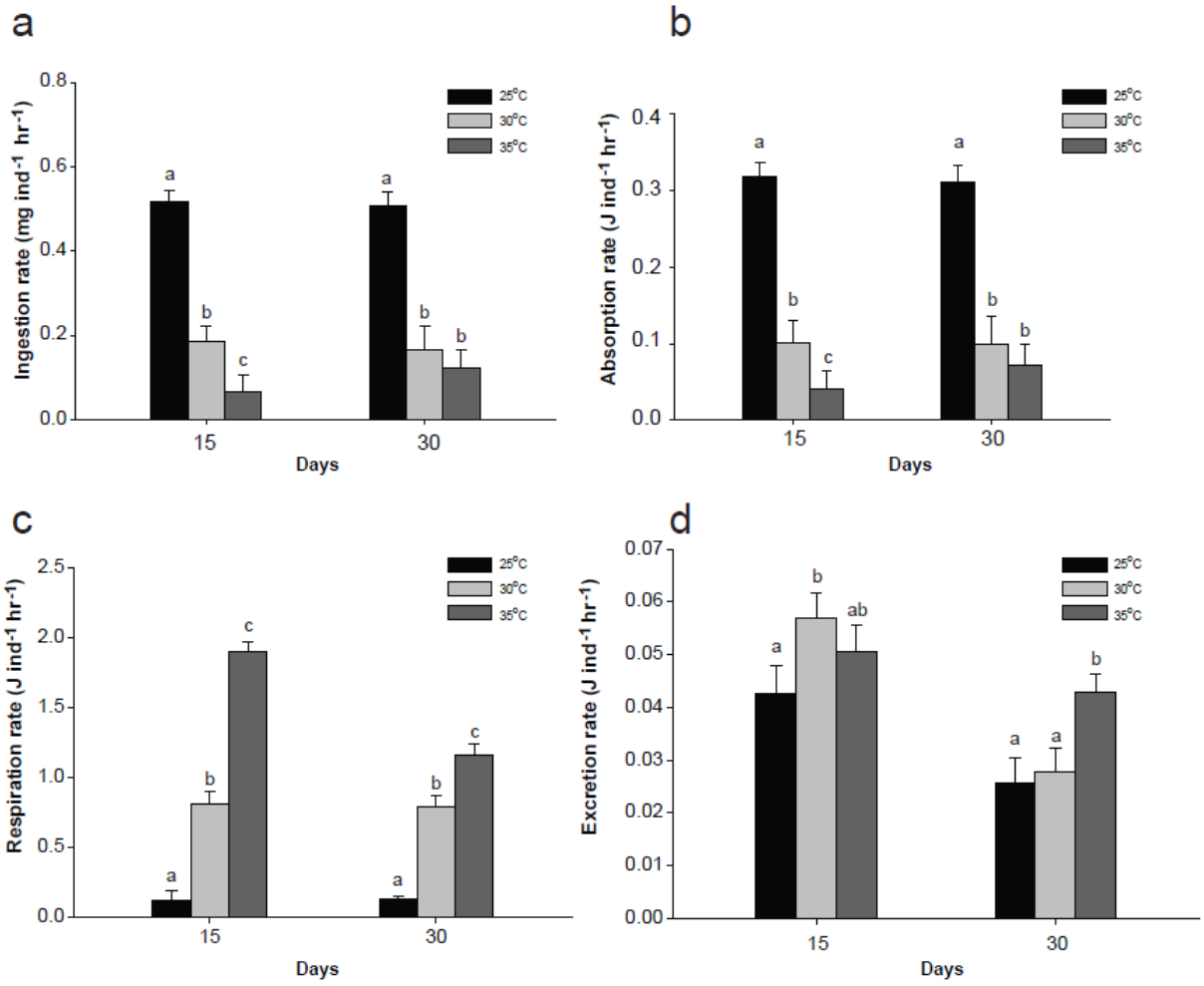


Fig.2. Ingestion rate (a), Absorption rate (b), Respiration rate (c), Excretion rate (d) of *Bellamya bengalensis* under different temperature treatments (25ºC, 30ºC and 35ºC) on 15th and 30th day of the experiment. Values represent mean $\pm$ SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

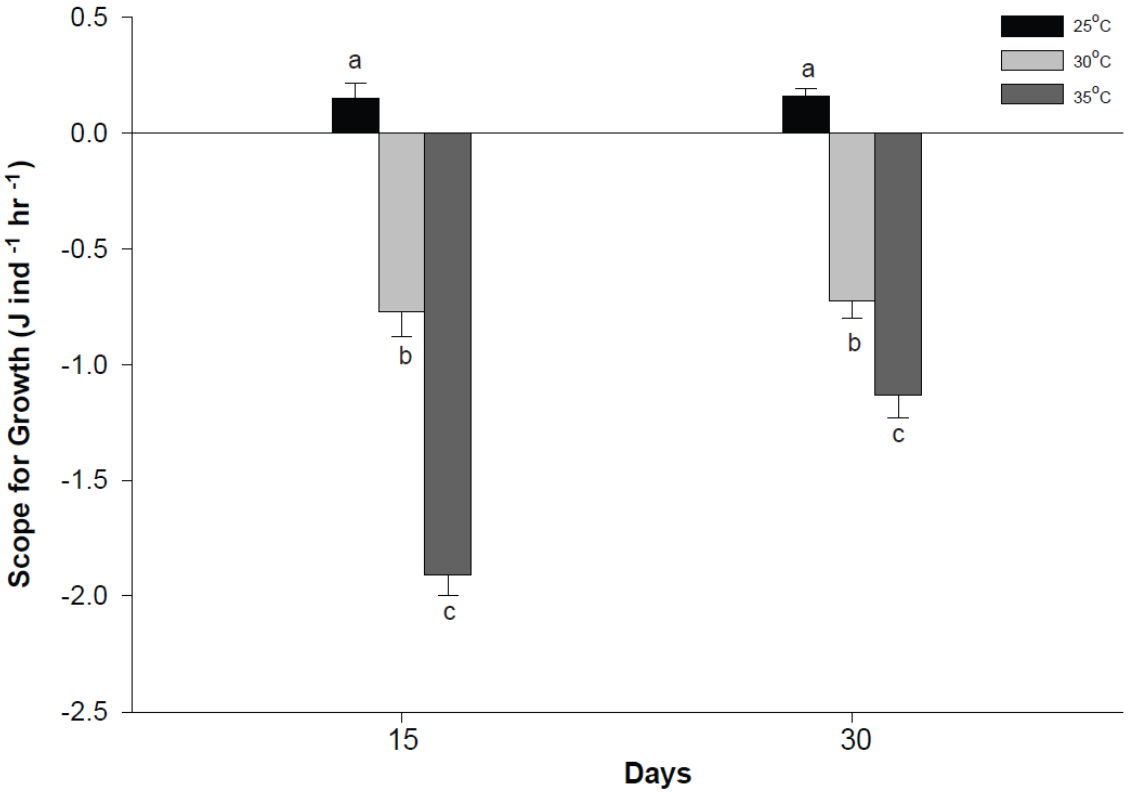


Fig.3. Scope for Growth of *Bellamya bengalensis* under different temperature treatments (25ºC, 30ºC and 35ºC) measured on 15th and 30th day of the experiment. Values represent mean $\pm$ SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

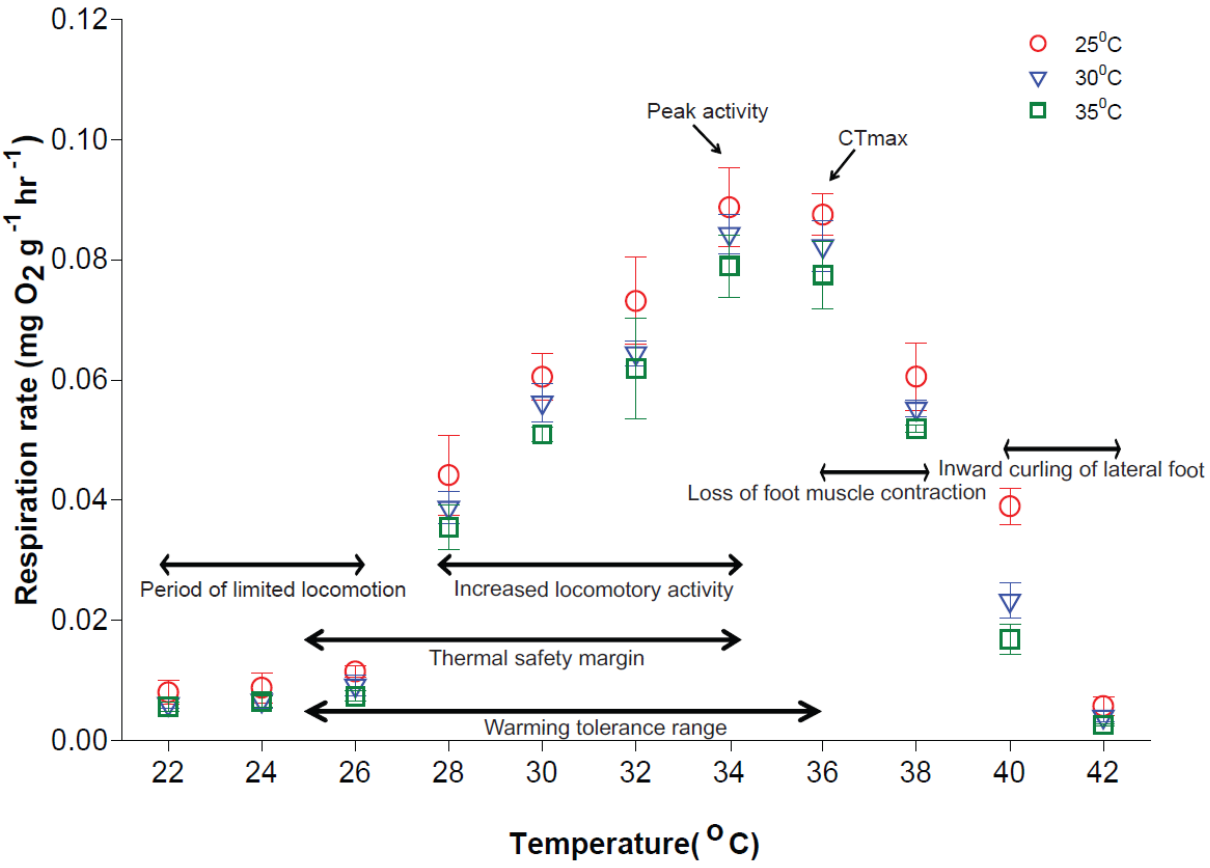


Fig.4. The thermal performance curve of *Bellamya bengalensis* along an increasing thermal ramp (+2°C hr^{-1}) from 22°C to 42°C after 31 days of exposure to different temperature treatments (25ºC, 30ºC and 35ºC) indicating CTmax (thermal critical maxima), ABT (Arrhenius breakpoint temperature), T_{hab} (average water temperature), WT (warming tolerance), TSM (thermal safety margin) values for all the treatment groups. Values represent mean ± SD (n = 5).

VI. Conclusion/ Discussion

The study established physiological adjustments in *Bellamya bengalensis* exposed to climate change mediated elevated temperature under its thermal tolerance boundary. The animals survive by reducing their stored energy reserves and compromising their energy budget. Elevated temperature exhibited a significant impact on the ingestion, absorption, respiration, excretion rates as well as Scope for growth in gastropods; yet, a very slow acclimatization process was noted which might require more time. The CTmax temperature indicated that the animals are already residing at their thermal tolerance boundary which might endanger their population in future. An increased water temperature for prolonged time leads to hypoxemia and deteriorate the systemic oxygen tensions. Furthermore, it may modify the set points of

acid-base equilibrium which can compress the thermal space and the optimal performance of this animal would eventually decline.

VII. Significance of the study

Present study highlights thermal stress significantly affect the physiology and growth in *Bellamya bengalensis* which may impede the freshwater ecosystem functioning in projected climatic scenario.

Experiment 2: (Objective 3) (Baag et al., 2021; Chemosphere)

IV. Methodology

The study discusses the effect of an environmentally pertinent dose of chlorpyrifos (organophosphate pesticide), under ambient (25°C) and higher levels of temperature (30°C, 35°C) in *Bellamya bengalensis* for 60 days across several endpoints. Gastropods were randomly distributed into experimental tank equally in three 10 L plastic aquaria (25 individual animals per replicate tank) for each treatment. Three different temperature regimes were tested (25°C, 30°C and 35°C) individually as well as combined with a pesticide dose of 0.40ng/ml. The pesticide dose of chlorpyrifos (0.40ng/ml) was chosen as the average pesticide concentration found in the adjacent areas of freshwater bodies (Mondal et al., 2018). Analytical standard grade chlorpyrifos was obtained from Sigma-Aldrich. Various levels of biomarkers were assessed: growth conditions (organ to flesh weight ratio, condition index), oxidative stress level (SOD, CAT, GST, LPO) as well as DNA damage (Comet assay-3^{rd}, 30^{th} and 60^{th} days only) after acute (24, 48 and 72 hours) and long-term exposures (10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th} and 60^{th} days). Finally, an integrated biomarker response (IBR) index was applied to amalgamate results generated from various biomarkers to assess organism's vulnerability to pesticide pollution and how it may shift with warming climate.

The hepatopancreatic tissue was dissected in cold tissue homogenizer holding phosphate buffer (pH 7.2, 0.1 M, 4°C) for studying the biomarkers. The tissue was then homogenized and centrifuged. The supernatant collected was utilized for the assays. Superoxide dismutase (SOD) activity was determined by calculating the rate of inhibition of hematoxylin autooxidation following to Martin et al. (1987). Catalase (CAT) activity was evaluated by spectrophotometric method and calculating the rate of disintegration of H_2O_2 the enzyme substrate (Aebi,1984). Glutathione-S-transferase activity was assessed according to Habig et al. (1974) by means of 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Lipid

peroxidation levels were determined by development of thiobarbituric acid reactive substances (TBARs) following Ohkawa et al. (1979). The results attained were normalized by total protein content estimated through the method of Bradford (Bradford, 1976). DNA damage assay was carried out by alkaline comet assay protocol adapted from the method described in Singh et al. (1988).

Integrated biomarker response (IBR) was estimated following Beliaeff and Burgeot (2002) with variations suggested in Devin et al. (2014). Individual biomarker response results were standardized by a formula $Y = (X - m)/s$, where Y denotes the standardized biomarker's response, X denotes the response of each biomarker at a particular time; m and s signify mean value and standard deviation, respectively. Z was then calculated as $Z = -Y$ or $Z = Y$, depending on the biological consequence respectively for an inhibition or induction. The scores (S) for the biomarker were calculated as $S = Z + |\text{Min}|$, where |Min| denotes the minimum value for individual biomarker estimated from the standardized biomarker response. The score results (S) were exhibited in star plots and were used to calculate the integrated biomarker response (IBR) as:

$$\text{IBR} = \sum_1^n Ai$$

$$A_i = S_i * S_{i+1} * \sin(2\pi/k)/2$$

where, A_i represents the triangular area denoted by two consecutive biomarker scores on the star plot, S_i and S_{i+1} signify the individual biomarker scores (calculated from standardized data) and their consecutive star plot radius coordinates, and k denotes the number of radii corresponding to the biomarkers used in the experiment.

Statistical analysis:

To investigate the effect of temperature, pesticide dose and duration of stress (number of days) individually as well as synergistically on growth conditions, biochemical and cellular biomarkers permutational analysis of variance (PERMANOVA) was applied. The main test was performed using temperature, pesticide dose and duration as fixed factors and whenever significant interaction were found between these factors, a paired test was performed for each factor separately in each level from other factor. All the analyses were performed in PRIMER 6 (Clarke and Gorley, 2006; Clarke et al., 2008) with PERMANOVA+ add on (Anderson et al., 2008).

V. Results

Significant variations were detected in growth conditions during longer experimental periods. Organ to flesh weight ratio diminished on 30th, 50th and 60th days in higher temperature treatments in contrast to control (Fig. 5a). No substantial effect of temperature was noted in the earlier exposure times but from 30th day onwards noteworthy variations were found in condition index (Fig. 5b). Acute along with long-term exposures intensified the antioxidant and detoxification enzyme activity. There were significant separate and combined effects of temperature, pesticide dose and time on SOD (Fig. 6a-c), CAT (Fig. 6d-f) and GST (Fig. 7a-c) activity. LPO levels varied significantly (Fig. 7d-f) in all treatments compared to control and were impacted by temperature, pesticide dose and time individually and interactively. Cellular level damage was not prevented by antioxidant and detoxification mechanisms. DNA damage was immense under prolonged exposure to stress however it was also considerably severe under acute extreme warming (Fig. 8a,b,c). IBR indicated prolonged acclimation in *B. bengalensis* to warming and pesticide contamination as depicted by improved tolerance however, acute stress was more deleterious (Fig. 9). Higher IBR response was noted on 3rd day in all the treatments excluding 30ºC in contrast to control (Table 1). Howbeit, during longer exposures low IBR values were noted in all temporal treatments in contrast to control. Amongst the treatment groups, the maximum IBR were noted in the 35ºC groups in all temporal treatments. Moreover, all the biomarkers exhibited a reduction in IBR among the treatments compared to control except few exceptions (Table 2).

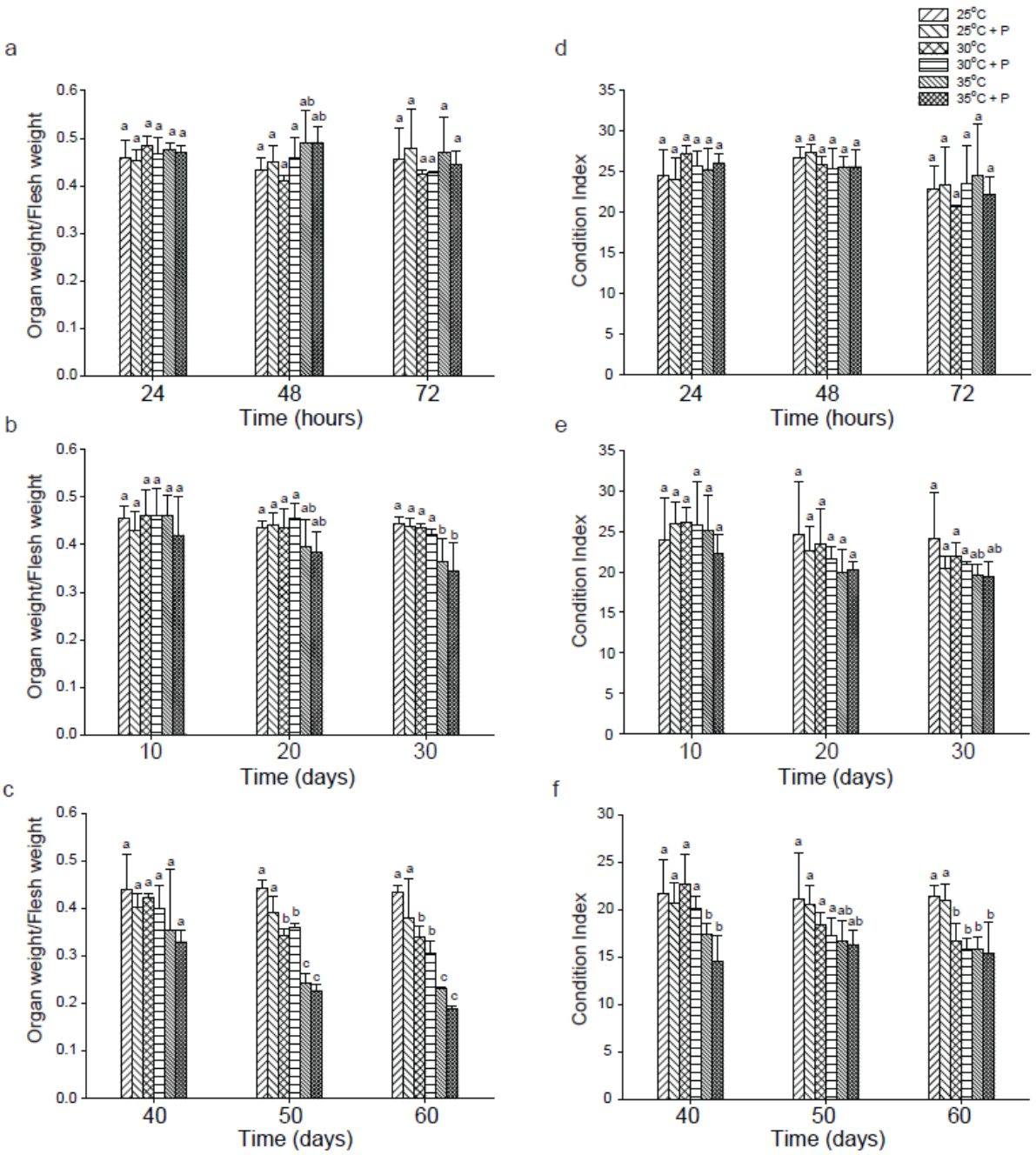


Fig.5. Changes in organ to flesh weight ratio on 24, 48, 72 hours (a) 10, 20, 30 days (b) 40, 50, 60 days (c) and condition Index on 24, 48, 72 hours (d) 10, 20, 30 days (e) 40, 50, 60 days (f) of *B. bengalensis* under different temperature and/or chlorpyrifos treatments during the experimental period. Values represent mean ± SD (n = 9, $p \leq 0.05$). Different letters indicate significant difference between temperature treatment groups (a, b, c) at each sampling time. No significant difference was found with respect to pesticide contamination.

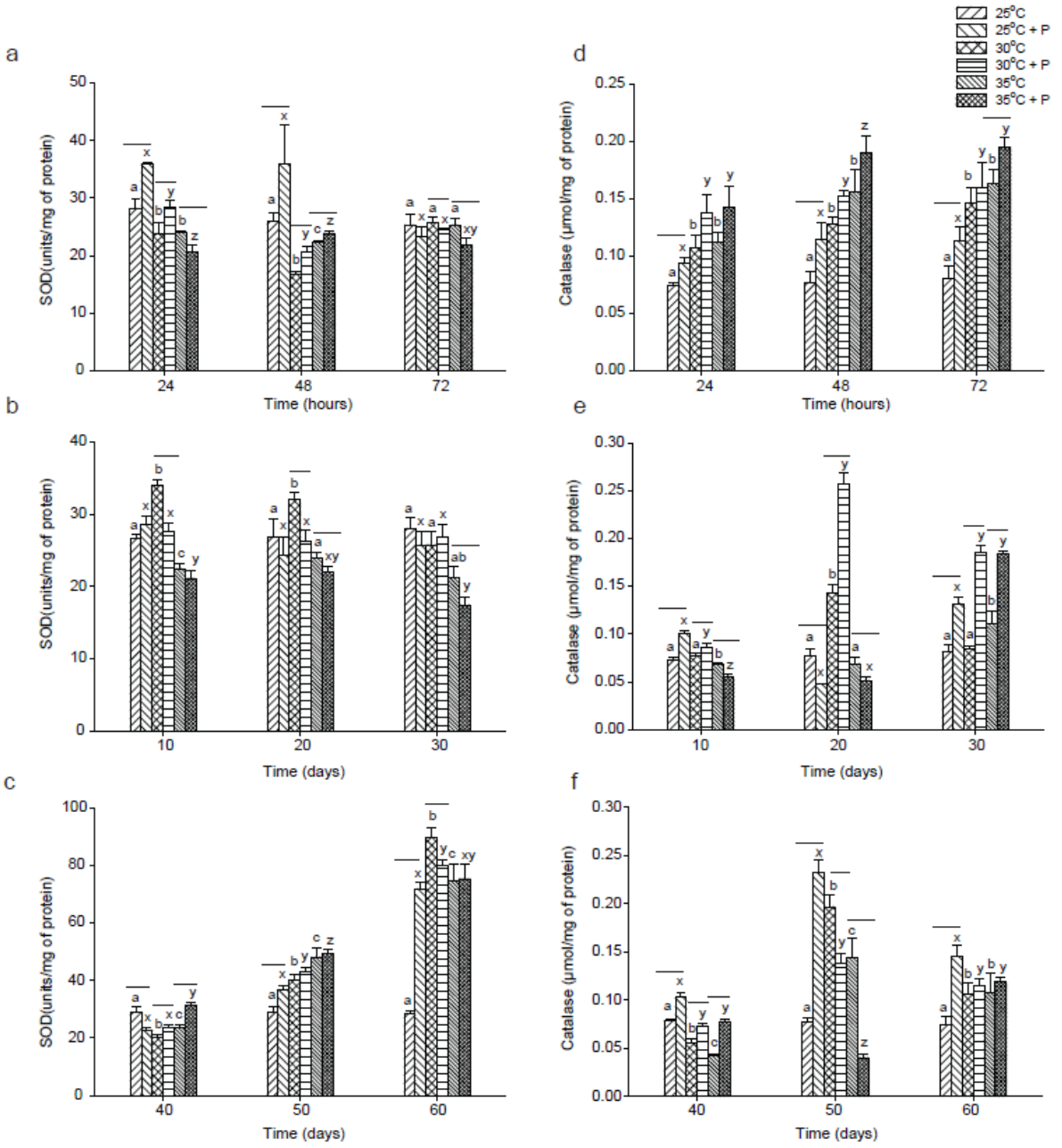


Fig.6. Changes in SOD activity on 24, 48, 72 hours (a) 10, 20, 30 days (b) 40, 50, 60 days (c) and CAT activity on 24, 48, 72 hours (d) 10, 20, 30 days (e) 40, 50, 60 days (f) of *B. bengalensis* under different temperature and/or chlorpyrifos treatments during the experimental period. Values represent mean ± SD (n = 9, $p \leq 0.05$). Different letters indicate significant difference between temperature treatment groups without pesticide contamination (a, b, c) and with pesticide contamination (x, y, z) at each sampling time. The means sharing the symbol " ¯ " is used to mark the significantly different pesticide treatment groups under the same temperature at each sampling time.

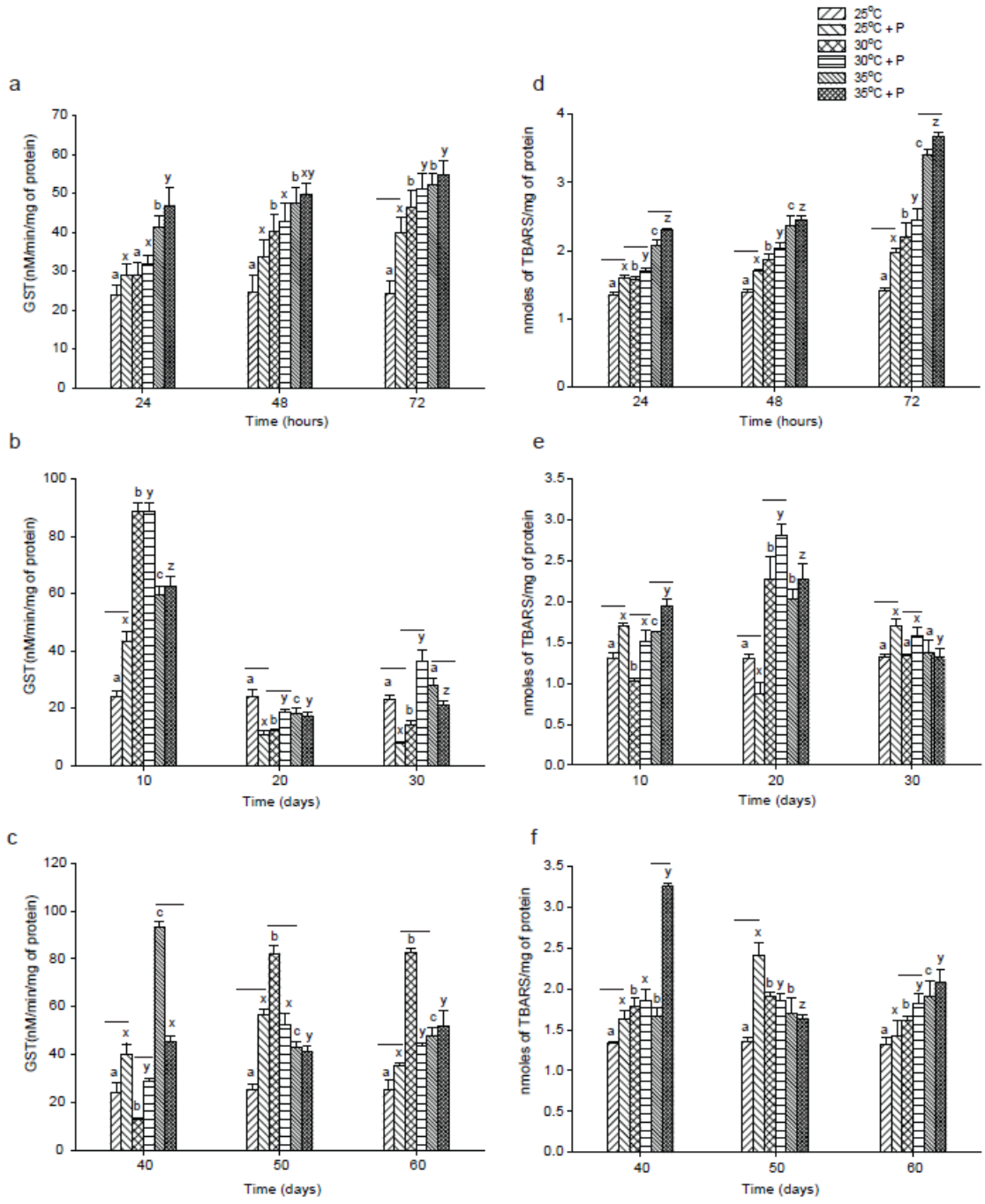
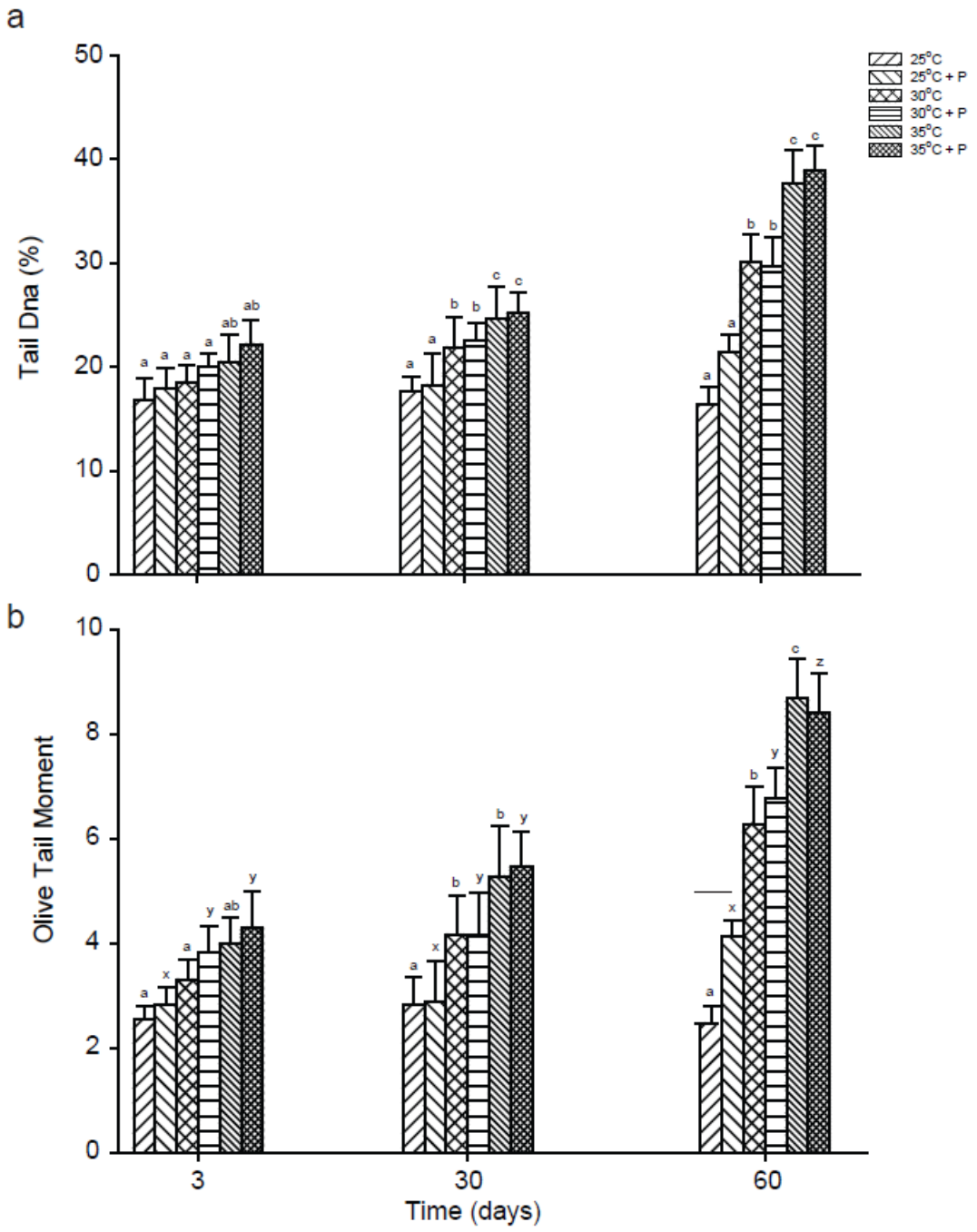


Fig.7. Changes in GST activity on 24, 48, 72 hours (a) 10, 20, 30 days (b) 40, 50, 60 days (c) and LPO levels on 24, 48, 72 hours (d) 10, 20, 30 days (e) 40, 50, 60 days (f) of *B. bengalensis* under different temperature and/or chlorpyrifos treatments during the experimental period. Values represent mean ± SD (n = 9, $p \leq 0.05$). Different letters indicate significant difference between temperature treatment groups without pesticide contamination (a, b, c) and with pesticide contamination (x, y, z) at each sampling time. The means sharing the symbol " ¯ " is used to mark the significantly different pesticide treatment groups under the same temperature at each sampling time.


a
b
Tail Dna (%)
Olive Tail Moment
Time (days)
3
30
60
25°C
25°C + P
30°C
30°C + P
35°C
35°C + P

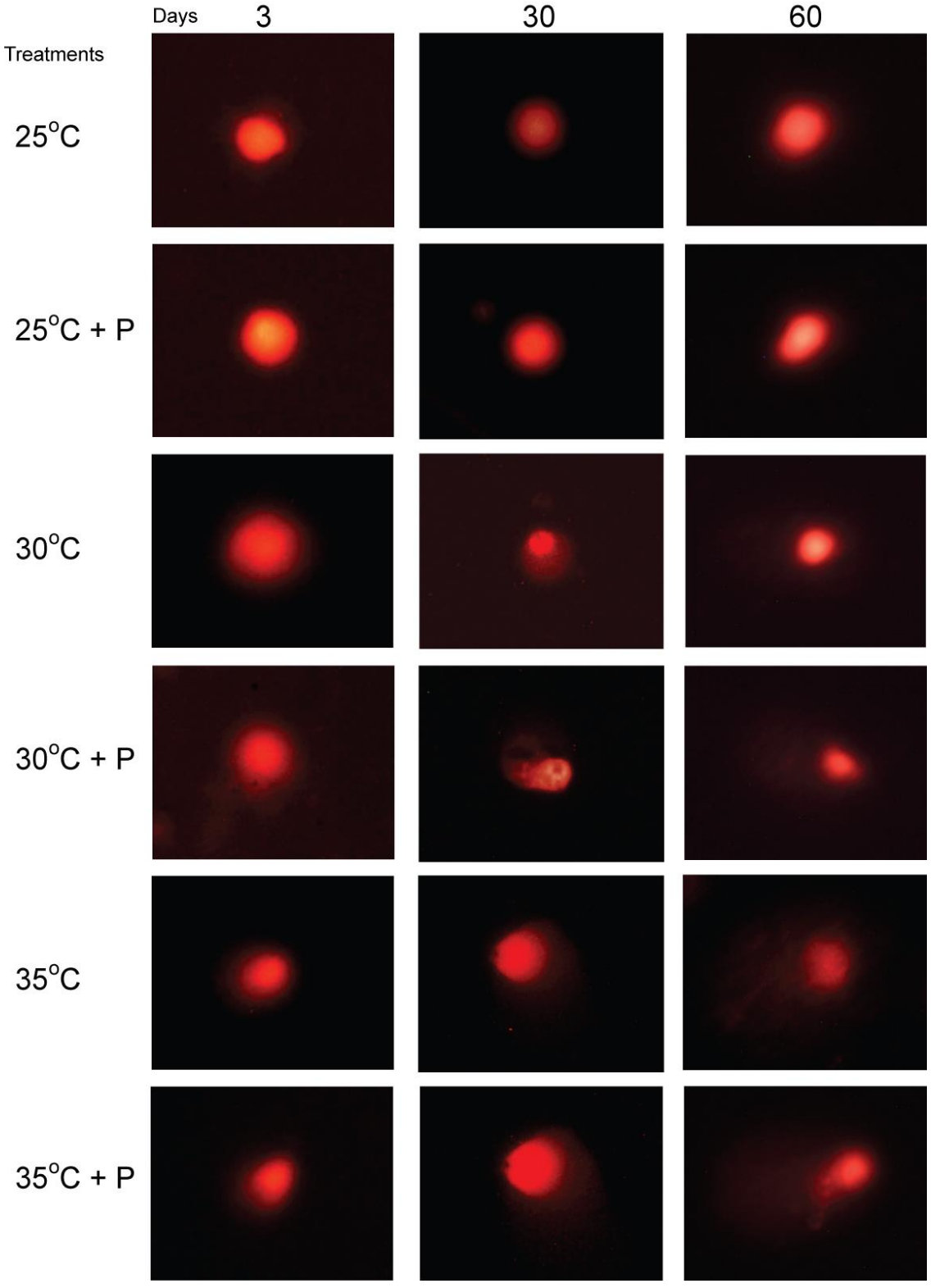


Fig.8. Changes in Tail DNA percentage (a) and Olive Tail Moment (b) on 3, 30 and 60 days of *B. bengalensis* under different temperature and/or chlorpyrifos treatments during the experimental period. Values represent mean ± SD (n = 6, $p \leq 0.05$). Different letters indicate significant difference between temperature treatment groups (a, b, c) at each sampling time for Tail DNA percentage. No significant difference was found with respect to pesticide

contamination for Tail DNA percentage. Different letters indicate significant difference between temperature treatment groups without pesticide contamination (a, b, c) and with pesticide contamination (x, y, z) at each sampling time for Olive Tail Moment. The means sharing the symbol " ¯ " is used to mark the significantly different pesticide treatment groups under the same temperature at each sampling time Olive Tail Moment. DNA damage, as measured by the comet assay in *Bellamya bengalensis* after staining with ethidium bromide at different temperature and pesticide treatments on 3rd, 30th and 60th days (c).

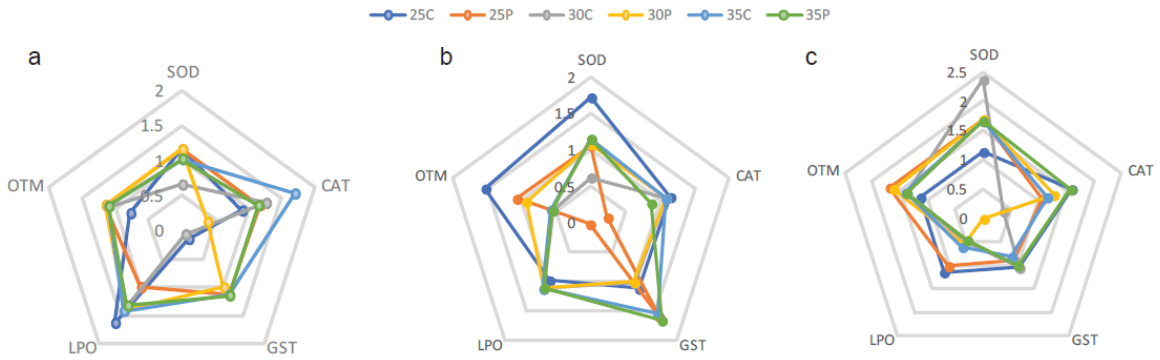


Fig.9. Star plots of standardized biomarker response (SOD, CAT, GST, LPO and OTM) in *B. bengalensis* exposed to different temperature and/or chlorpyrifos treatments for 3 days (a), 30 days (b) and 60 days (c).

Table 1	Time of exposure		
Treatments	3rd Day	30th Day	60th Day
25˚C	1.72	4.01	4.25
25˚C + P	3.01	0.87	3.89
30˚C	1.53	1.82	2.72
30˚C + P	2.45	2.58	2.77
35˚C	3.24	3.79	2.83
35˚C + P	3.14	2.61	3.62

Table 1. Integrated biomarker response (IBR) values in *B. bengalensis* exposed to elevated temperature and chlorpyrifos contamination. IBR values were calculated at different times of exposure taking into account several stress biomarkers- SOD, CAT, GST, LPO and OTM.

Table 2	Treatments					
Biomarkers	25˚C	25˚C + P	30˚C	30˚C + P	35˚C	35˚C + P
SOD	2.49	2.35	1.62	2.39	2.26	2.27
CAT	2.04	0.84	1.05	1.08	2.38	1.98
GST	0.71	2.02	0.58	0.49	1.89	2.24
LPO	2.18	0.5	1.28	1.42	1.49	1.23
OTM	1.81	2.37	1.37	2.11	1.43	1.39

Table 2. Integrated biomarker response (IBR) values for different stress biomarkers- SOD, CAT, GST, LPO and OTM in *B. bengalensis* exposed to elevated temperature and chlorpyrifos contamination. For each biomarker, the IBR value was calculated taking into account different times of exposure (3, 30 and 60 days).

VI. Conclusion/ Discussion

The experiment demonstrated combined impact of temperature elevation and organophosphate pesticide contamination on multiple biomarkers of *Bellamya bengalensis*. A good interpretation of the relations amongst the different biomarkers and treatments was facilitated by the IBR index which provided a holistic idea of the cellular damage caused by these factors. This work confirms the utility of multiple biomarker approach to evaluate the toxicity in *B. bengalensis* and advocates the practicality of using it in biomonitoring studies as a model organism. Moreover, considering multiple endpoints and their progress over prolonged time (acute and long-term exposures) can aid in clarification of the probable mechanisms underneath the mutual action of the stressors applied. This study affirms the vulnerability of the studied gastropod to extreme climatic catastrophes like occurrence of sudden heatwaves, although might display resilience if there is slow change in the climatic patterns.

VII. Significance of the study

This study affirms the utility of using multiple biomarkers to evaluate the toxicity in *B. bengalensis* and promotes the usefulness of this species and these methods for biomonitoring programmes. Since *B. bengalensis* is a major important component of the aquatic food chains in this area, its diminished population would imply a probable irreversible damage to the higher trophic level animals, including fish, birds and mammals.

Experiment 3: (Objective 1&2) (Baag and Mandal, 2023a; Environmental Science and Pollution Research)

IV. Methodology

The main purpose of the study was to understand whether rearing in global environmental drivers (ocean acidification and warming) aggravates the impact of a local driver (oil spill) on the energy budget of mud crab (*Scylla serrata*) from Sundarbans estuarine system. Adult *Scylla serrata*, (carapace length: 63.41±4.08mm, weight: 48.35±4.79g) were collected from the tidal creek of river Matla in Sundarbans. The crabs were fed with dried fish pellets till satiation 120 crabs were kept separately for 30 days under two scenarios (60 crabs in each scenario, 10 crabs in each aquaria): a) present scenario (pH 8.1, 28°C) and b) predicted climate change scenario for 2100 according to IPCC (2019) (pH 7.7, 34°C). The target temperatures were attained by immersing digitally controlled thermostats in water baths

holding experimental aquaria for the entire experimental duration. Seawater acidification scenario was mimicked using a pH stat system (Cole-Parmer, USA). The pH values were set and controlled by means of a computerized system connected to individual pH probes with an automatic cut off mechanism controlling the CO_2 gas flux. After this experimental exposure period (30 days) half (30 crabs) of each treatment were exposed to 5mg L^{-1} of marine diesel oil (MDO) for 24hours (maintaining same water quality parameters for each treatment group) and the other half remained uncontaminated. The oil concentration was chosen to match a realistic oil spill scenario based on previous studies from different geographic locations (Bechmann et al. 2010; Sagerup et al. 2016; Mansour et al. 2017; Arnberg et al. 2019) and also from present geographic location (Ghosh and Mandal 2021). Marine diesel oil (MDO) is a blending of gasoil and heavy fuel oil used in marine diesel engines. MDO was purchased from a standard local fuel station near to fishing port for our experiment. This resulted in 4 different treatments (triplicate aquaria per treatment with 10 crabs per aquaria i.e., 30 crabs per treatment): 1) pH 8.1 and 28°C in the absence of MDO (considered as control condition in the experiment); 2) pH 8.1 and 28°C with MDO; 3) lower pH 7.7 and elevated temperature 34°C in the absence of MDO and 4) pH 7.7 and 34°C with MDO. Physiological functions (ingestion, absorption, respiration and excretion rates and Scope for Growth), thermal performance, thermal critical maxima (CTmax), acclimation response ratio (ARR), Arrhenius activation energy (AAE), temperature coefficient (Q_{10}) warming tolerance (WT), as well as thermal safety margin (TSM) were assessed.

Methodology followed same as Experiment 1

Statistical analysis

The effect of climate change (CC) stressors (ocean warming and acidification) and oil as well their interaction was investigated on ingestion rate, assimilation efficiency, absorption rate, respiration rate, excretion rate, scope for growth. A two-way per mutational analysis of variance (PERMANOVA) was applied. The main test was performed using climate change stressors and oil as fixed factors and whenever significant interaction were found between these factors, a paired test was conducted for each factor separately in each level from other factor. The effect of climate change factors was also assessed by PERMANOVA on the thermal performance of the test species. All the analyses were performed in PRIMER 6 (Clarke and Gorley 2006; Clarke et al. 2008) with PERMANOVA + add on (Anderson et al. 2008).

V. Results

Ingestion as well as absorption rates were significantly declined by the effect of climate change factors and oil as well as their interaction (Fig. 10a&b). Substantial alterations were found amongst all the treatments. Within stress treatments, maximum ingestion and absorption were noted in the OAW treatment (lower pH 7.7 and elevated temperature 34°C in the absence of MDO), while the least in the combined OAW and oil treatment (pH 7.7 and 34°C with MDO). Respiration rate (RR) also significantly varied with climate change factors, oil and their interaction (Fig. 10c). A significant variation between all the treatment groups was noted. RR declined significantly after exposure to MDO in the current climatic situation (pH 8.1 and 28°C with MDO). Howbeit, RR increased significantly in OAW conditions irrespective of the presence or absence of MDO in contrast to the control conditions. The maximum RR was noted in the OAW treatment group in the absence of MDO. In all the stress treatment groups the excretion rates were considerably high in comparison to control animals (Fig. 10d). Scope for growth (SfG) significantly reduced in *S. serrata* under MDO exposure irrespective of the climatic treatments they were exposed (Fig.11). Although diminished, SfG was maximum in the OAW treatment in the absence of MDO amongst all stress treatments in comparison to the control. The cross-stress treatment displayed significant exacerbated effect of stressors with the lowermost SfG amid all the treatments. A significant effect on thermal performance in stress acclimated treatment group was also observed, leading to a lower CTmax value (Fig. 12). A decrease in CTmax was also noted in cross-stress group. CTmax reached at 36°C for stress-acclimated animals, while in the control animals CTmax arrived at 38°C. Thus, CTmax arrived 2°C earlier in stressed crabs than the control crabs indicating a reduced thermal tolerance limit. The acclimation response ratio (ARR) was estimated to be 0.33. The warming tolerance levels were 10°C and 8°C; though, thermal safety margins were 8°C and 6°C for control and stressed animals, respectively.

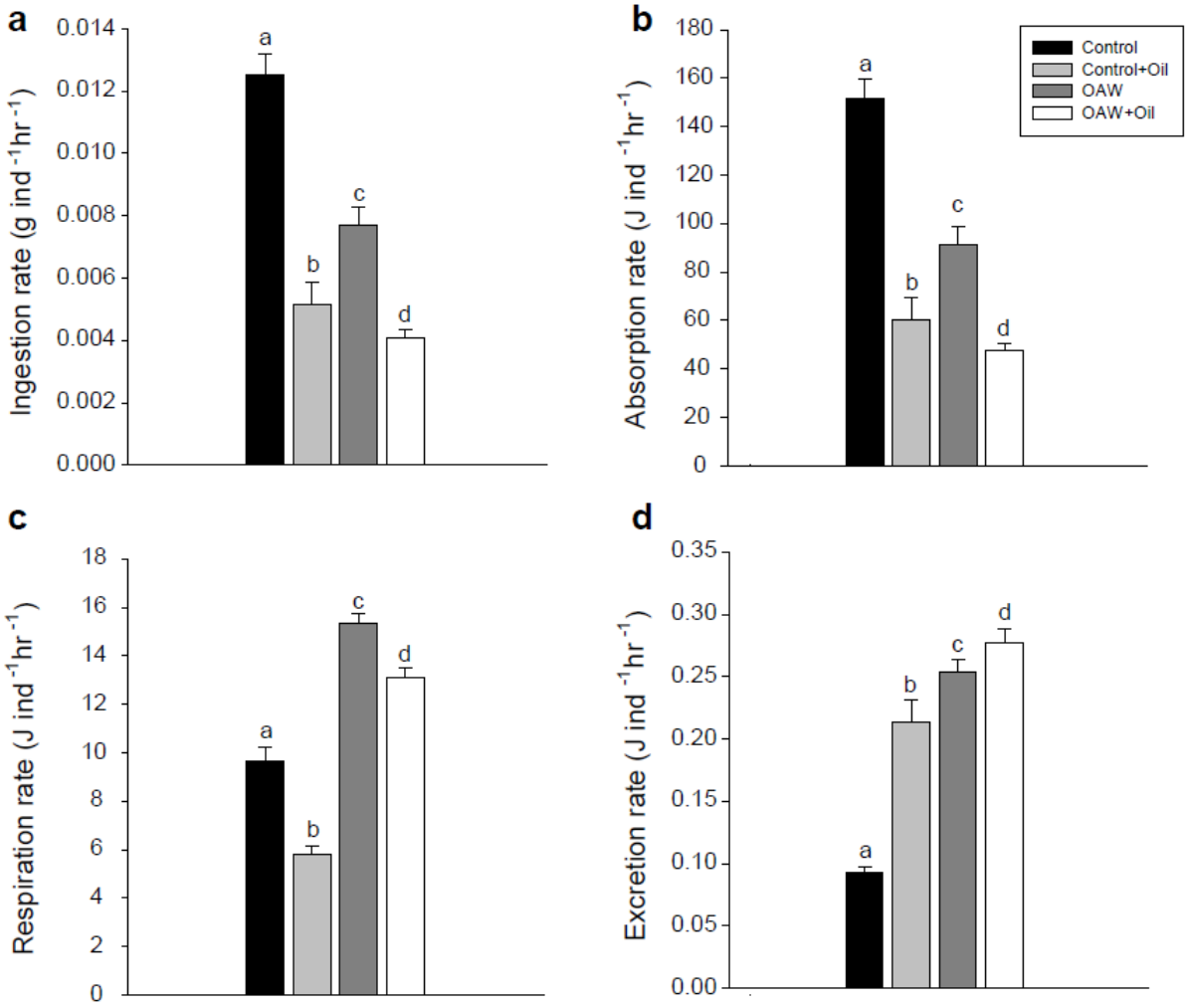


Fig. 10. Ingestion rate (a), Absorption rate (b), Respiration rate (c), Excretion rate (d) of *Scylla serrata* under different treatments of the experiment. Values represent mean ± SD (n=9, $p< 0.05$). Different letters (a, b, c, d) indicate significant difference between treatment groups.

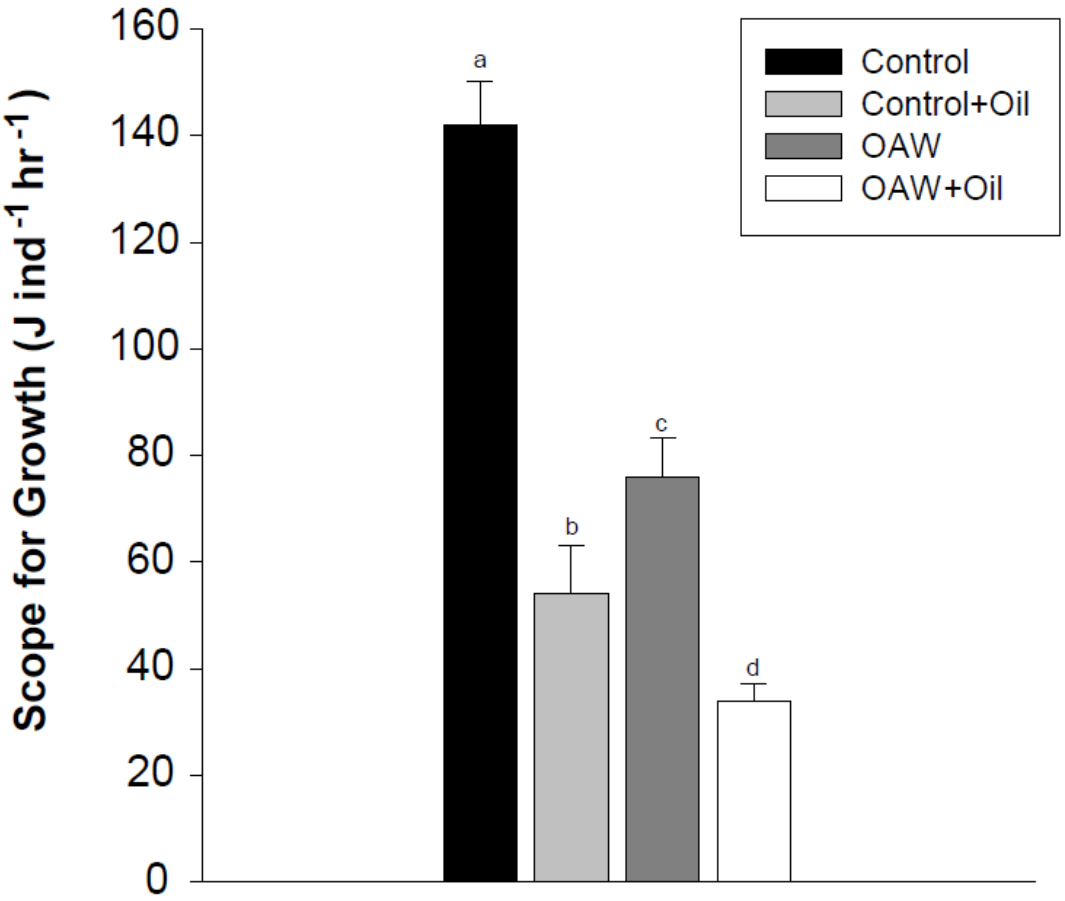


Fig. 11. Scope for Growth of *Scylla serrata* under different treatments of the experiment. Values represent mean ± SD (n=9, $p< 0.05$). Different letters (a, b, c, d) indicate significant difference between treatment groups.

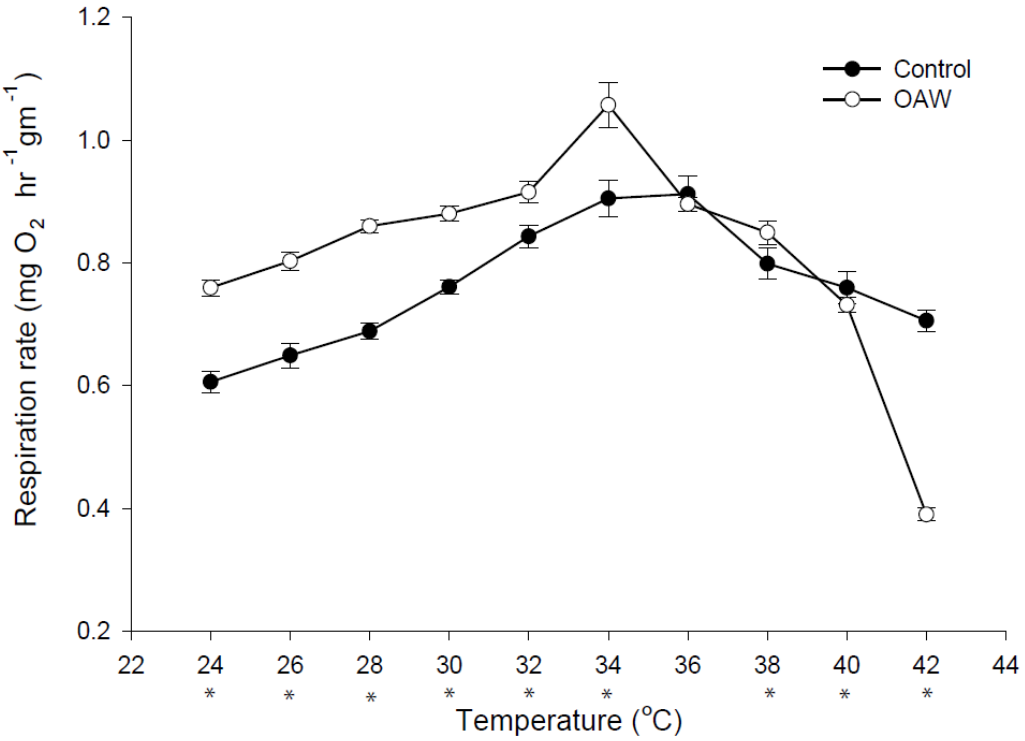


Fig. 12. The thermal performance curve of *Scylla serrata* along an increasing thermal ramp (+2°C hr^{-1}) from 24°C to 42°C after 30 days of exposure to different treatments indicating CT_{max}. Values represent mean ± SD (n=6). The temperature levels marked with "*" indicate a significant difference between the two acclimated groups.

VI. Conclusion/ Discussion

This study established that combined impacts of global environmental factors ocean acidification warming (OAW) intensifies the effect of acute oil pollution (spill) and reduce the energy budget in mud crab *S. serrata*. Accessible energy is vital in shaping the adaptive mechanisms of marine animals under shifting oceanic climate. Suboptimum environmental conditions disturb the energy budget which result in diminished energy left for adaptive responses under added stress, ultimately sensitizing animals to pollutants. This study confirmed that OAW conditions constricts the tolerance potential as well as increase the energy expenditure requisite for homeostasis in this species. Integrated effects of environmental stress alongside pollution exhibited lower energy budget at the individual level which in turn may cause a repercussion in the future population level. This detrimental result indicates a confined geographical distribution of this animal in forecasted climate change conditions.

VII. Significance of the study

This study substantiates that the integrated effects of global environmental factors' ocean acidification and warming aggravates the effect of acute oil spill by decline in the energy budget and constricting the thermal tolerance capacity of *Scylla serrata* that may potentially diminish their population and disturb coastal aquaculture in impending years. The present work highlights the importance of environmentally pertinent multiple stressors in toxicological research. The results obtained accentuate the role of natural stressors in enhancing pollutant's toxicity towards aquatic animals.

Experiment 4: (Objective 3) (Baag and Mandal, 2023b; Comparative Biochemistry and Physiology B)

IV. Methodology

The key objective of the study was to decipher whether exposure to ocean warming and acidification intensifies the effect of pollution (oil spill) on oxidative stress status of *S. serrata*. 120 animals were treated for 30 days under two scenarios separately (60 crabs in each scenario, 10 crabs in each aquaria): a) current environmental scenario (pH 8.1, 28°C) and b) projected future climate change scenario for 2100 (pH 7.7, 34°C). After this experimental duration, 50% animals (30 crabs) from each treatment were exposed to 5mg L^{-1} of marine diesel oil (MDO) for 24hours. Oxidative stress parameters like superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), lipid peroxidation (LPO level) and DNA damage (Comet assay) were analysed. Integrated biomarker response (IBR) index was used to integrate results produced across the different biomarkers.

Methodology followed same as Experiment 2

Statistical analysis

Permutational analysis of variance (PERMANOVA) was applied to investigate the effects of environmental factors and oil exposure individually and together on multiple biomarkers. The main test was conducted using environmental scenario and oil exposure as fixed factors. Only whenever the main test revealed statistical significance ($p \leq 0.05$), pairwise evaluations were performed.

V. Results

Amplified antioxidant and detoxifying enzyme activity was noticed (excluding SOD) which failed to counterbalance LPO and DNA damage. A substantially increased SOD activity (Fig. 13a) was observed at only oil treatment of control condition whereas in case of the climate change (CC) condition the activity significantly diminished irrespective of oil exposure in comparison to the control. CAT and GST activity significantly increased in all the treatments when compared to control (Fig. 13b&c). Higher LPO levels were also noted in all treatment groups in contrast to control (Fig. 13d). Treatment contaminated with oil in both environmental conditions induced noteworthy LPO levels with the highest in combined exposure (pH 7.7 and 34˚C with MDO). Tail DNA percentage significantly increased in both climatic conditions irrespective of oil contamination (Fig. 14a,b,c). IBR confirmed that OAW conditions reduce the tolerance potential of crabs to acute oil pollution. IBR results exhibited SOD and CAT to be the most responsive whereas GST and LPO to be the least responsive biomarkers in this species (Fig. 15a&b).

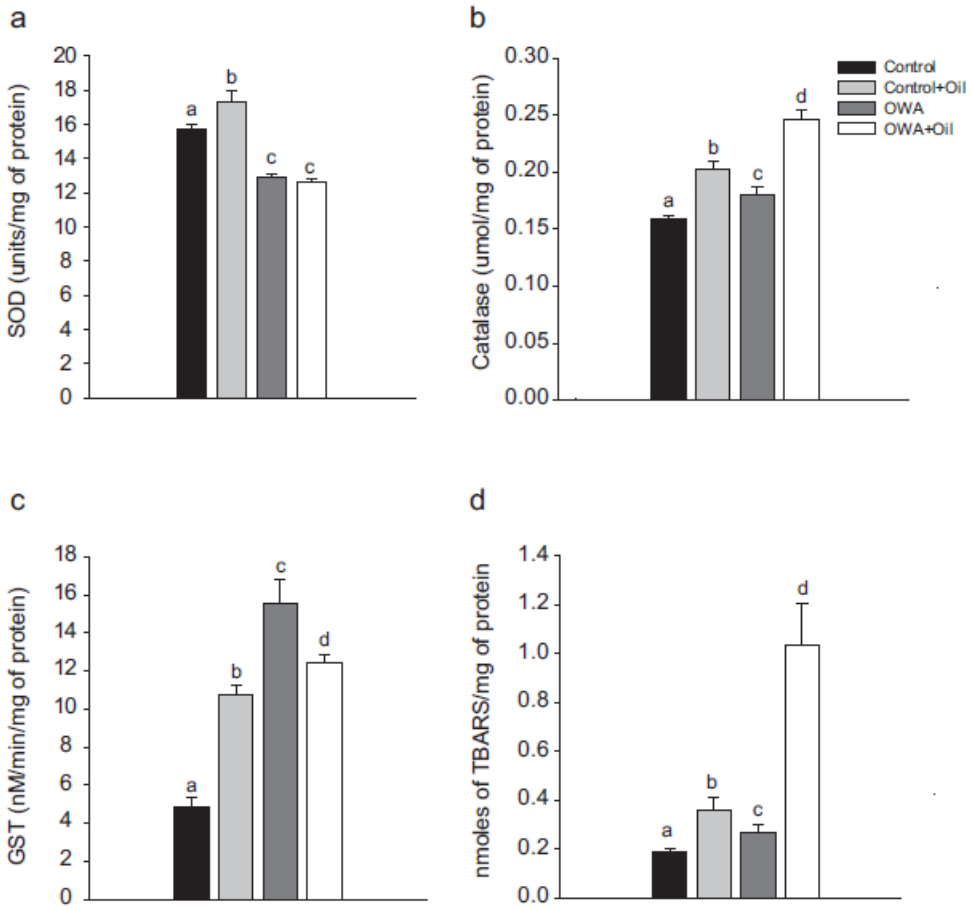
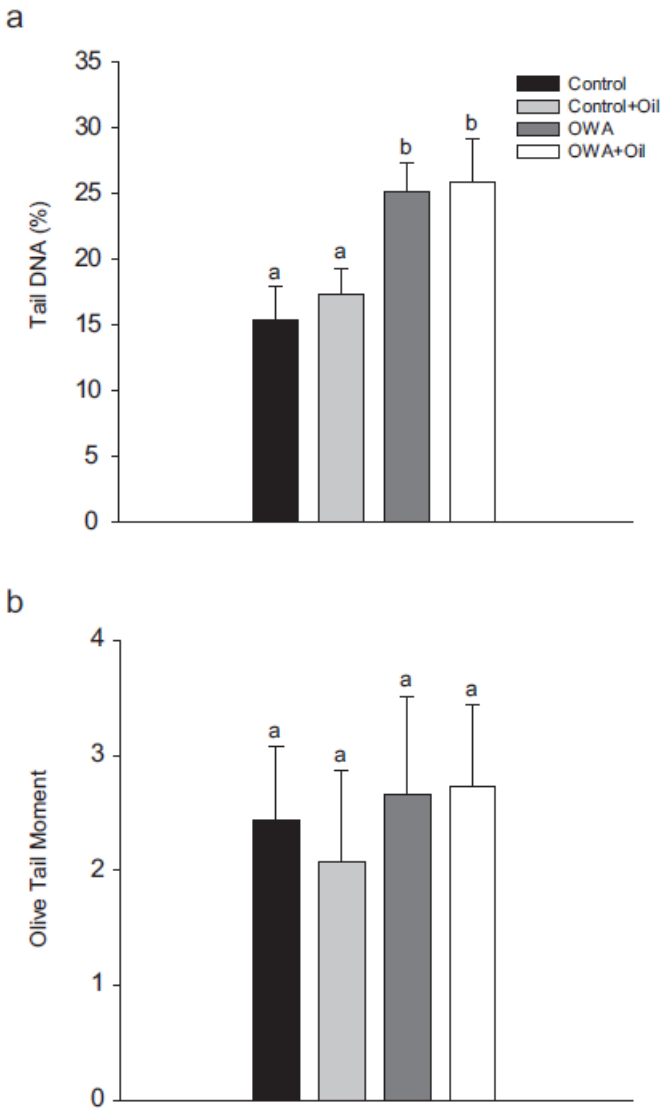


Fig. 13. Changes in SOD activity (a), CAT activity (b), GST activity (c), LPO levels (d) of *Scylla serrata* under different treatments of the experiment. Values represent mean ± SD

(n=9, $p < 0.05$). Different letters (a, b, c, d) indicate significant difference between treatment groups.



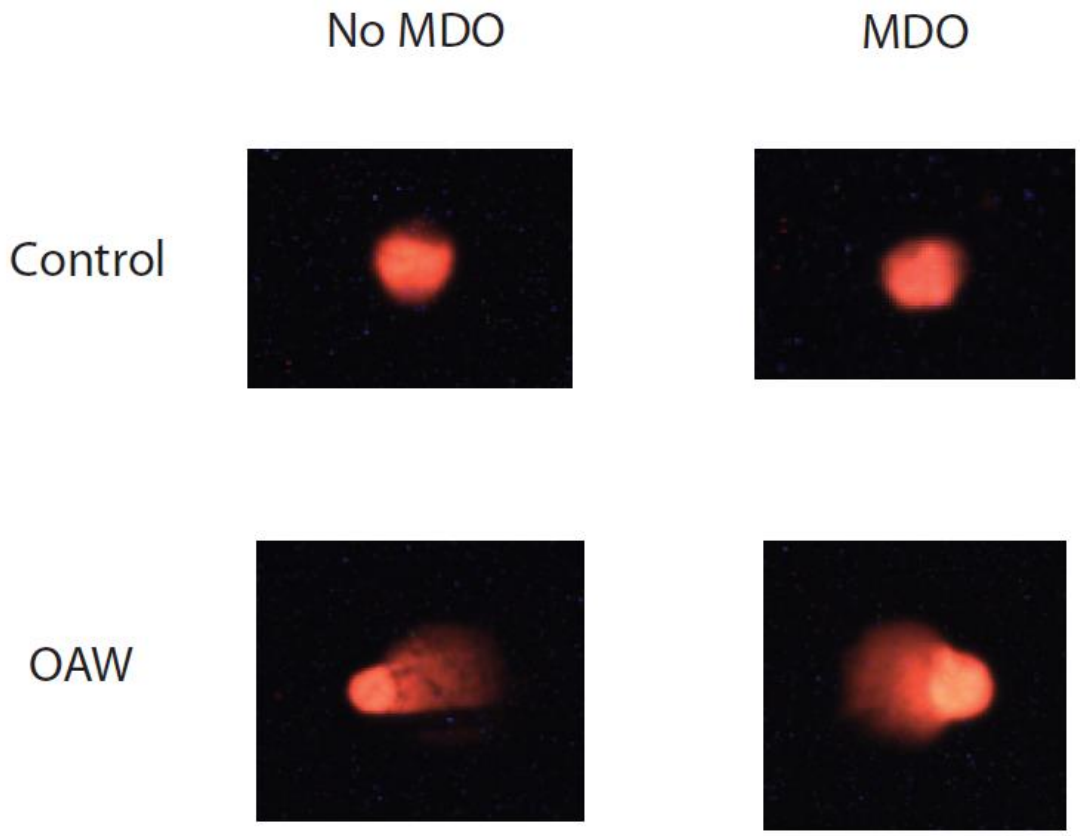


Fig. 14. Changes in Tail DNA percentage (a) and Olive Tail Moment (b) of *Scylla serrata* under different treatments of the experiment. Values represent mean ± SD (n=6, $p< 0.05$). Different letters (a, b, c, d) indicate significant difference between treatment groups. DNA damage, as measured by the comet assay in *Scylla serrata* after staining with ethidium bromide at different treatments (c).

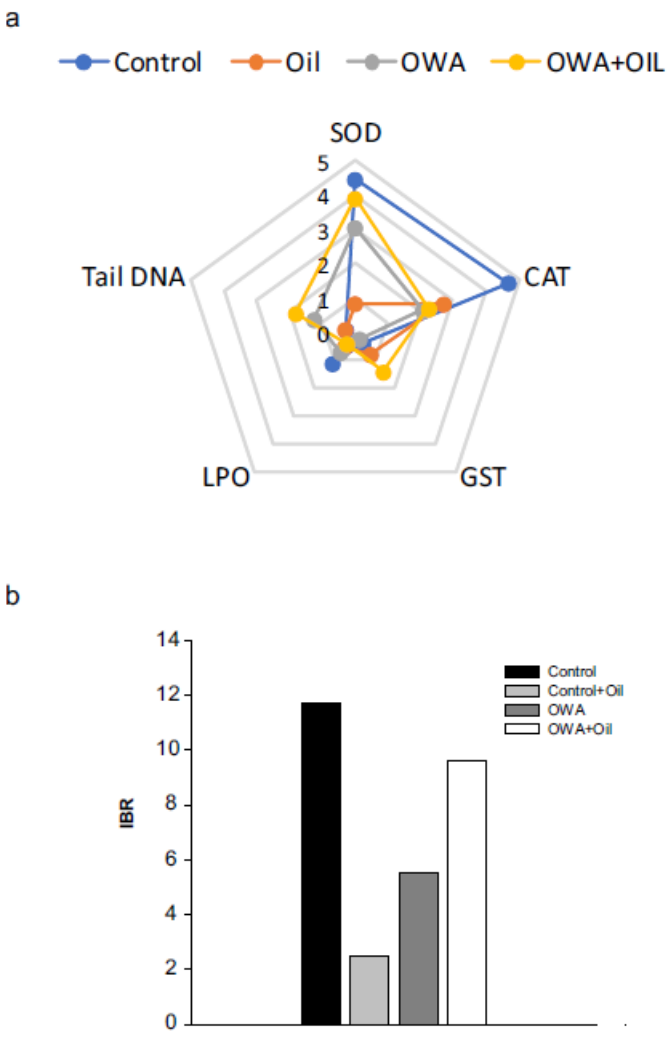


Fig. 15. Star plots representing standardized biomarker response (SOD, CAT, GST, LPO and Tail DNA) (a), Integrated biomarker response (IBR) score for different stress treatments in *Scylla serrata* exposed to different treatments (b).

VI. Conclusion/ Discussion

The current study offers idea about the antioxidant defence system of crabs exposed in acute oil pollution following acclimation in ocean acidification and warming conditions. The results advocate that future climatic conditions will exacerbate the impact of acute pollution and induce oxidative stress causing DNA damage in *S. serrata*. Integrated biomarker responses enhanced our concept of the antioxidant response mechanism in multiple stressor conditions in contrast to discrete biomarkers in isolation.

VII. Significance of the study

This study offers understanding of the antioxidant defence system of crabs subjected to acute oil pollution after exposure to combined effects of ocean acidification and warming conditions. With the anticipated risk of OAW and oil spill pollution in the study area, the *S. serrata* population of might experience regional extinction in case of lack of resilience capacity which might significantly jeopardize the local aquaculture in Sundarbans.

Experiment 5: (Objective 1&3) (Baag et al. under review in Comparative Biochemistry and Physiology C)

IV. Methodology

The prime aim of the investigation was to decipher the toxicity of Zinc oxide nanoparticles (ZnO-NP) after simultaneous exposure to a global environmental stressor, elevated temperature for 14 days and followed by a 7 days recovery period, on the physiological responses of mud crab *Scylla serrata* from Sundarbans estuarine system. The crabs were treated under 6 treatments- two temperature (28ºC and 34ºC) and three concentrations of ZnO-NP (0, 1, and 5 mg L^{-1}) for 14 days which yielded six different treatments a) 28ºC, WNP (without Nanoparticle) regarded as control, b) 28ºC, 1mg L^{-1} NP, c) 28ºC, 5mg L^{-1} NP, d) 34ºC, WNP, e) 34ºC, 1mg L^{-1} NP, f) 34ºC, 5mg L^{-1} NP. Each treatment had 3 replicates holding 10 crabs in each replicate aquaria. The temperatures were chosen to match present average habitat temperature of the crabs (28ºC) and predicted future temperature by 2100 (34ºC) (IPCC, 2019). The NP concentrations used in the experiment were within the range commonly used in laboratory experiments to match realistic environmental scenario (Huang et al., 2018). The exposure period (14 days) was followed by a recovery period of 7 days without ZnO-NP and at control temperature 28ºC. The total experimental duration was 21 days.

Physiological energetics such as ingestion, assimilation, absorption, respiration, and excretion rates were measured to determine the Scope for growth (SfG). Additionally, we assessed various biomarkers (antioxidant, detoxification defence mechanisms, and lipid peroxidation levels) of the species.

Methodology followed same as Experiment 1&2 combined.

Statistical analysis

Permutational analysis of variance (PERMANOVA) was conducted to examine the effects of elevated temperature and nanoparticle contamination independently as well as in combination on physiological variables and various biomarkers. The main test was executed using elevated temperature and nanoparticle contamination as fixed factors. When the main test indicated statistical significance ($p \leq 0.05$), pairwise tests were executed.

V. Results

Elevated temperature, nanoparticle contamination and their interaction significantly impacted ingestion, absorption, respiration and excretion rates in crabs (Fig. 16a-d). Combined stress attenuated the SfG in crabs which further deteriorated in the recovery phase (Fig. 17). Oxidative stress was also exacerbated under coalesced stress condition. SOD, CAT, GST activity remarkably varied with elevated temperature and nanoparticle contamination as well their interaction (Fig. 18a-c). Recovery was not observed in crabs with increased lipid peroxidation level under combined stress conditions (Fig. 18d). Elevated temperature disturbed the energy budget of crabs as mirrored by diminished energy left for compensatory responses under additional metal stress, eventually sensitizing the animals to ZnO NP pollutants.

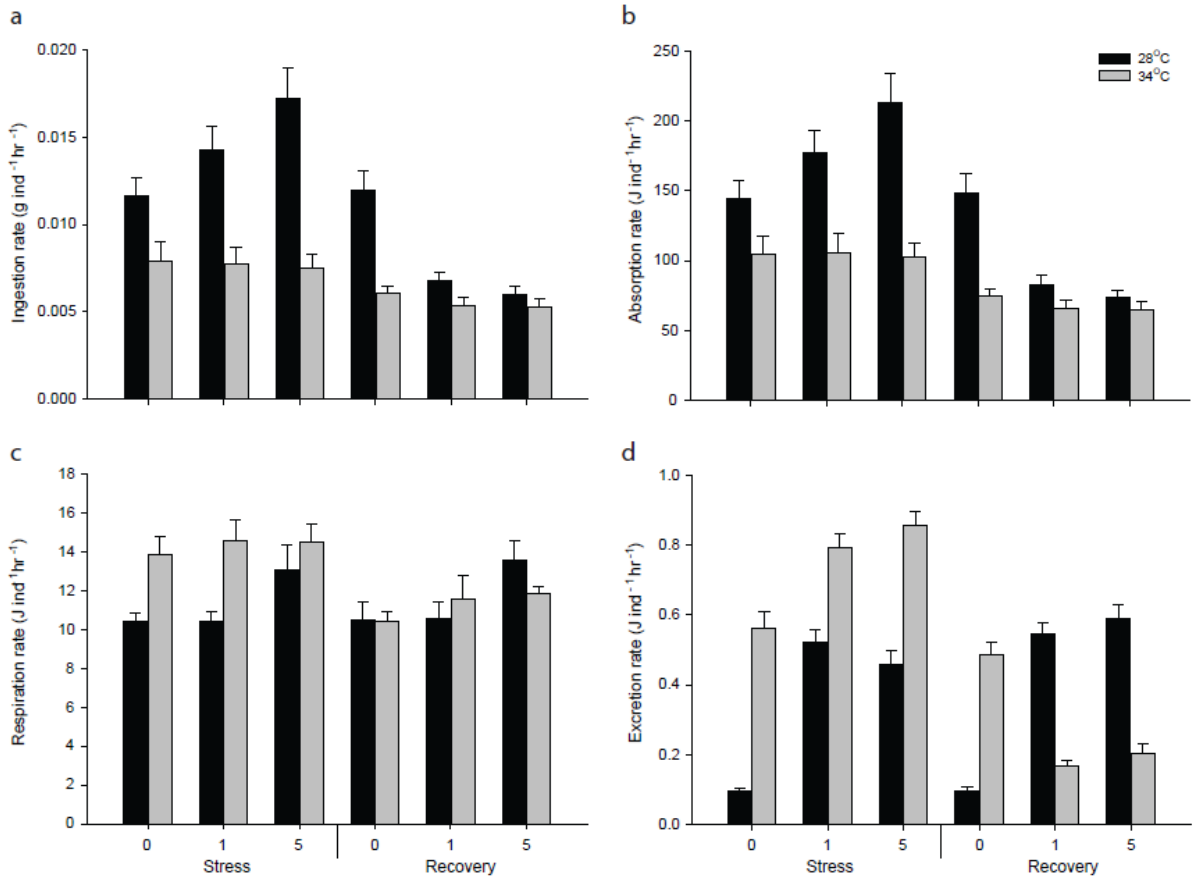


Fig. 16. Changes in Ingestion rate (a), absorption rate (b), respiration rate (c), excretion rate (d) of *Scylla serrata* under different stress and recovery treatments of the experiment. 0, 1, 5 represents the concentration of ZnO NP in mg L^{-1}. Values represent mean ± SD (n = 9, $p < 0.05$).

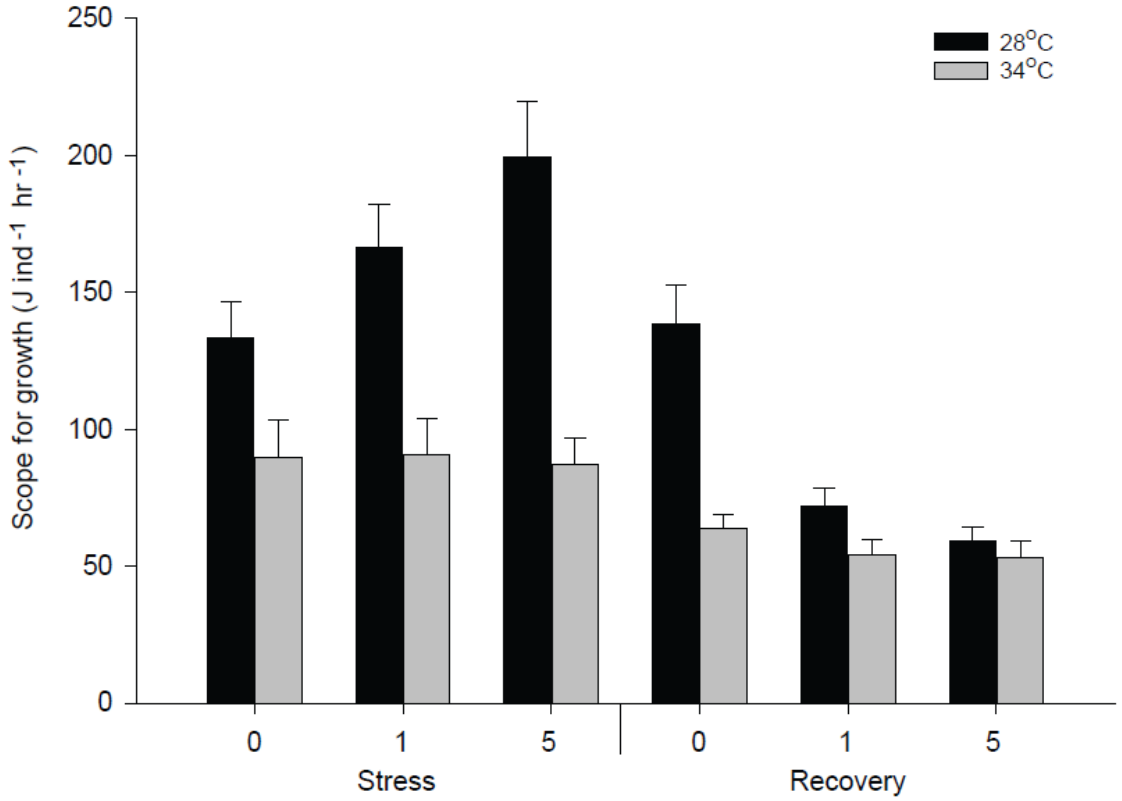


Fig. 17. Changes in Scope for growth of *Scylla serrata* under different treatments of *Scylla serrata* under different stress and recovery treatments of the experiment. 0, 1, 5 represents the concentration of ZnO NP in mg L^{-1}. Values represent mean ± SD (n = 9, $p < 0.05$).

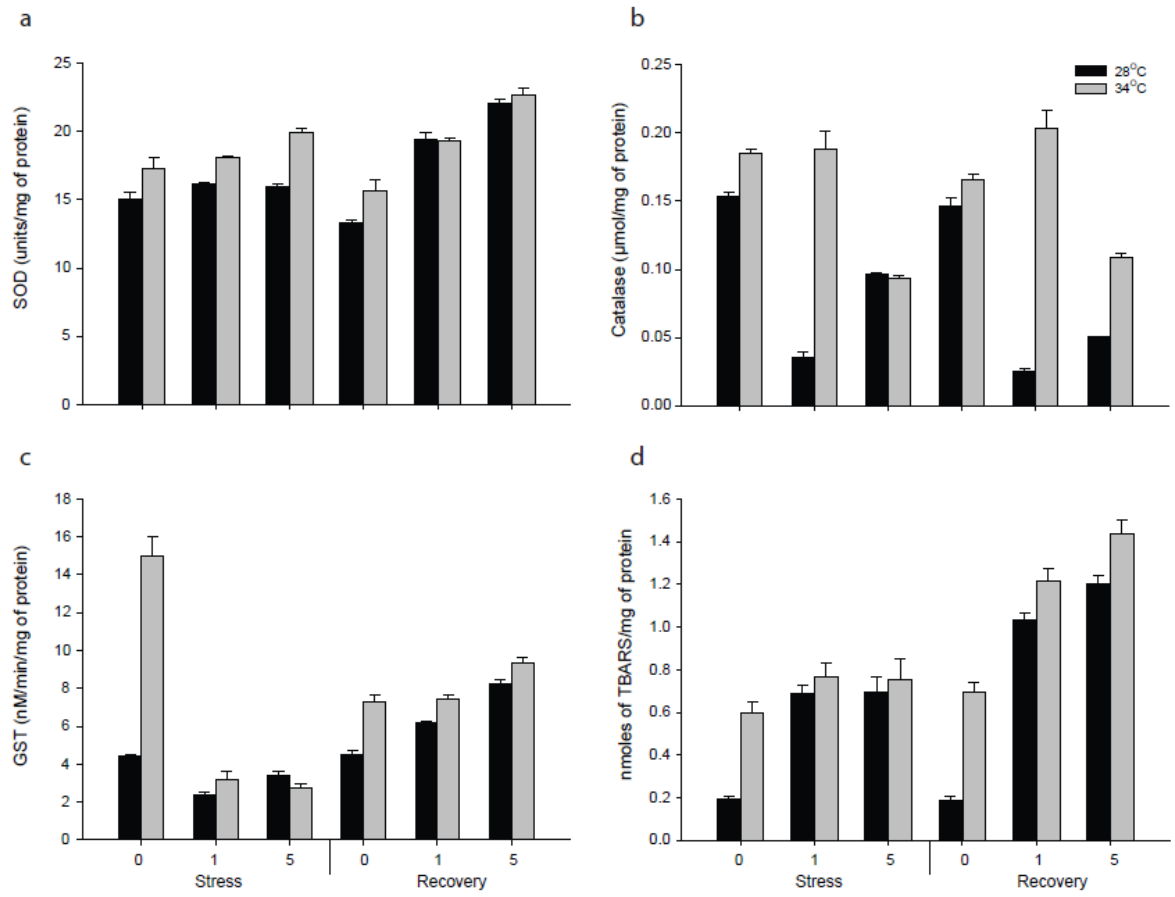


Fig. 18. Changes in SOD activity (a), CAT activity (b), GST activity (c), LPO levels (d) of *Scylla serrata* under different stress and recovery treatments of the experiment. 0, 1, 5 represents the concentration of ZnO NP in mg L^{-1}. Values represent mean ± SD (n = 9, $p < 0.05$).

VI. Conclusion/ Discussion

The study assessed the influence of elevated temperature and exposure to Zn nanoparticles on stress response and recovery potential of crabs *S. serrata.* The energy availability is vital to ascertain the adjustment potential of marine organisms under future oceanic situations. In our study elevated temperature disturbed the energy budget of crabs resulting in diminished energy left for compensatory responses under additional metal stress and eventually sensitizing the animals to ZnO NP pollutants. The present results also suggests that future ocean temperature is expected to exacerbate the effects of metal NP pollution and cause oxidative damage in *S. serrata*. Both the physiological and biochemical results depicted that combined stress from elevated temperature and nanoparticle contamination restrained the

recovery in crabs. The current results also emphasize the ability of natural stressors in intensifying a pollutant's toxicity to estuarine species.

VII. Significance of the study

The present results imply future ocean temperature to exacerbate the effects of metal NP pollution and cause oxidative damage in *S. serrata*. Elevated temperature disturbed the energy budget of crabs resulting in diminished energy left for compensatory responses under additional metal stress and eventually sensitizing the animals to ZnO NP pollutants. Nanoparticle contaminated crabs could possibly jeopardize marine ecosystem functioning and dwindle financial growth through fishery sector in forthcoming years if exposed to elevated temperature simultaneously.

VIII. Future Directions

From the past few decades marine ecosystems are experiencing several environmental problems and innumerable anthropogenic disturbances. Only a few countries have given attention to this aspect and research works are still inadequate to deliver a holistic picture. The effects of multiple drivers are reliant upon ecological and evolutionary changes. Fluctuations in these drivers will impose direct or indirect repercussions on fish and shellfish health, their numbers and distribution pattern, immunity, predation success and competition. Hitherto, most experimental studies have concentrated on only short-term work and single species. Future global research should emphasize on effects of climate change at community level responses, as response from a single species are not likely to deliver adequate information to recognize functional complexity in ecosystems. We must also remember that apart from global climatic factors few regional biotic and abiotic components might also play a determining role in imminent aquatic ecosystems. Researches merging climatic factors and emerging chemical pollutants are required to better comprehend and understand biological effects in the future ocean. This would aid in developing significant ideas to compare new observance. Multi generation studies must be emphasized to better understand the evolutionary significances of these drivers and accumulate knowledge about their resilience capacity in future, though might be a daunting task. Marine ecosystem researches cannot anymore focus on individual stressor studies, as multiple interactive stress factors will control the ecosystem functioning. It is recommended to combine monitoring in situ as well as in laboratory (experimental) studies. Organisms residing at highly shifting natural habitats should be studied more to gain clear knowledge on the impacts of climatic stress on these species and their habitat. Ecological pyramids and food chains components should be taken into consideration to better comprehend the adaptive capacity of ecosystems to global

changes. Ecosystem approach to aquaculture (EAA) should be prioritized to promote sustainable aquaculture in an integrated manner.

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Appendix:

AAE: Arrhenius activation energy

Ab: Absorption rate

ABT: Arrhenius breakpoint temperature

AE: Assimilation Efficiency

ARR: Acclimation response ratio

CAT: Catalase

CTmax: Thermal critical maxima

GST: Glutathione S-transferase

LPO: lipid peroxidation

OAW: Ocean acidification warming

OTM: Olive Tail Moment

Q_{10}: Temperature coefficient

ROS: Reactive oxygen species

SfG: Scope for growth

SOD: Superoxide dismutase

TSM: Thermal safety margin

WT: Warming tolerance

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Unravelling the effects of elevated temperature on the physiological energetics of *Bellamya bengalensis*

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ABSTRACT

Temperature is one of the key environmental factors affecting the eco-physiological responses of living organisms and is considered one of the utmost crucial factors in shaping the fundamental niche of a species. The purpose of the present study is to delineate the physiological response and changes in energy allocation strategy of *Bellamya bengalensis*, a freshwater gastropod in the anticipated summer elevated temperature in the future by measuring the growth, body conditions (change in total weight, change in organ to flesh weight ratio), physiological energetics (ingestion rate, absorption rate, respiration rate, excretion rate and Scope for Growth) and thermal performance, Arrhenius breakpoint temperature (ABT), thermal critical maxima (CTmax), warming tolerance (WT) as well as thermal safety margin (TSM) through a mesocosm experiment. We exposed the animals to three different temperatures, 25 °C (average habitat temperature for this animal) and elevated temperatures 30 °C, 35 °C for 30 days and changes in energy budget were measured twice (on 15th and 30th day). Significant changes were observed in body conditions as well as physiological energetics. The total body weight as well as the organ/flesh weight ratio, ingestion followed by absorption rate decreased whereas, respiration and excretion rate increased with elevated temperature treatments resulting in a negative Scope for Growth in adverse conditions. Though no profound impact was found on ABT/CTmax, the peak of thermal curve was considerably declined for animals that were reared in higher temperature treatments. Our data reflects that thermal stress greatly impact the physiological functioning and growth patterns of *B. bengalensis* which might jeopardize the freshwater ecosystem functioning in future climate change scenario.

1. Introduction

Ever since the Industrial revolution, anthropogenic activities have tremendously increased green-house gases emission and caused global warming (Feely et al., 2004). It is now equivocally accepted that heat-waves are one of the pervasive consequences of global warming which are predicted to intensify in duration and frequency due to extreme climatic events, imposing elevated thermal stress (IPCC, 2013). Temperature elevation is a major concern in the scenario of global climate change which is a crucial environmental factor affecting the eco-physiology of aquatic ectotherms (Huey and Kingsolver, 1989; Hochachka and Somero, 2002). It has direct impact on metabolic rates, growth and reproduction and eventually on its survival (Van der Have and De Jong, 1996; Porter and Gates, 1969; Dallas and Rivers-Moore, 2012).

Invertebrates are broadly distributed throughout the ecosystems and can be used as excellent bio-monitors in ecosystem functioning studies (Lagadic and Caquet, 1998). In ectotherms, body temperatures are determined by interaction with environmental conditions. It is also a well-established fact that the tropical invertebrates are more vulnerable as they are already residing close to their optimal thermal edge (Deutsch et al., 2008). According to thermal tolerance paradigm, ectothermic species are highly regulated by subtle change in the environmental temperature for their survivability and performance attributes (Angilletta et al., 2002; Somero, 2010). The continuous physiological stress might limit the survival of such organisms and those possessing the ability to persist the stress with proper metabolic strategies would survive in future climate change scenario. Moreover, the acquired homeostatic capability would ultimately lead to decreased sensitivity due to prolonged thermal stress (Seebacher et al., 2015).

The potentiality to adjust with severe environmental changes is a key to survive and is brought about by comprising aerobic scope in aquatic ectotherms. Scope for Growth (SfG) is a sensitive physiological indicator which evaluates the instantaneous energy status of a particular animal

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An Integrated and Multibiomarker approach to delineate oxidative stress status of *Bellamya bengalensis* under the interactions of elevated temperature and chlorpyrifos contamination

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HIGHLIGHTS

- Results deciphered a significant effect of warming than pesticide contamination.
- Acute warming reflected vulnerability towards extreme climatic events.
- Long term studies reveal a scope for resilience against applied stressors.

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ABSTRACT

Synergistic effects of warming on bioconcentration and receptiveness of pollutants are still poorly unravelled in conjunction with cellular and molecular responses. The present study addressed the impact of an environmental relevant dose of chlorpyrifos (organophosphate pesticide), under control (25 °C) and elevated levels of temperature (30 °C, 35 °C) in *Bellamya bengalensis*, a freshwater gastropod for 60 days across various endpoints. Multiple levels of biomarkers were measured: growth conditions (organ to flesh weight ratio, condition index), oxidative stress status (SOD, CAT, GST, LPO) and DNA damage (Comet assay-3rd, 30th and 60th days only) after acute (24, 48 and 72 h) and long-term exposures (10th, 20th, 30th, 40th, 50th and 60th days). An integrated biomarker response (IBR) strategy was adapted to amalgamate results generated from various biomarkers to assess organism's vulnerability to pesticide pollution and how it may shift with warming climate. Significant changes were observed in growth conditions under longer exposure periods. Acute as well as long-term exposures enhanced the antioxidant and detoxification enzyme activity. DNA damage was extensive under longer exposure to stress howbeit was also significantly escalated under acute severe warming. Antioxidant and detoxification mechanisms fell short in counteracting cellular level damage. The IBR results indicated long-term acclimation of *B. bengalensis* to elevated temperatures and pesticide contamination lead to an improved tolerance level howbeit, acute stress was more detrimental. This study provided evidence for the efficiency of employing an integrated biomarker approach for *B. bengalensis* in future bio-monitoring studies.

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1. Introduction

Freshwater bodies are under constant threat of anthropogenic pressure primarily due to their proximity to human habitation. This imposes serious deleterious effects or alterations on the biochemical processes in the biota (Davies and Vethaak, 2012). Simultaneously, unprecedented rise of greenhouse gas emissions is predicted to occur till end of the 21st century (IPCC, 2007) and is regarded as one of the most crucial contributors of global warming, concomitantly increasing the average surface water temperatures on a global scale (IPCC, 2007). The capability of an animal to persevere the global climate change is entirely dependent on their resilience to adapt the swift warming scenario (Schiedek et al., 2007). Furthermore, temperature escalation may also alter animal's sensitivity towards other contaminants (Attig et al., 2014; Coppola et al., 2017; Andrade et al., 2019). Extensive use of pesticides in contemporary agricultural system has a remarkable contribution in polluting aquatic bodies across the world; and

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RESEARCH ARTICLE

Do global environmental drivers' ocean acidification and warming exacerbate the effects of oil pollution on the physiological energetics of *Scylla serrata*?

Sritama Baag[1] · **Sumit Mandal**[1]

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Abstract
Global climate change–induced ocean warming and acidification have complex reverberations on the physiological functioning of marine ectotherms. The Sundarbans estuarine system has been under threat for the past few decades due to natural and anthropogenic disturbances. In recent years, petroleum products' transportation and their usage have increased manifold, which causes accidental oil spills. The mud crab (*Scylla serrata*) is one of the most commercially exploited species in the Sundarbans. The key objective of this study was to delineate whether rearing under global environmental drivers (ocean acidification and warming) exacerbates the effect of a local driver (oil pollution) on the physiological energetics of mud crab (*Scylla serrata*) from the Sundarbans estuarine system. Animals were reared separately for 30 days under (a) the current climatic scenario (pH 8.1, 28°C) and (b) the predicted climate change scenario (pH 7.7, 34°C). After rearing for 30 days, 50% of the animals from each treatment were exposed to 5 mg L^{-1} of marine diesel oil for the next 24 h. Physiological energetics (ingestion rate, absorption rate, respiration rate, excretion rate, and scope for growth), thermal performance, thermal critical maxima (CT_{max}), acclimation response ratio (ARR), Arrhenius activation energy (AAE), temperature coefficient (Q_{10}), warming tolerance (WT), and thermal safety margin (TSM) were evaluated. Ingestion and absorption rates were significantly reduced, whereas respiration and ammonia excretion rates significantly increased in stressful treatments, resulting in a significantly lower scope for growth. A profound impact on thermal performance was also noticed, leading to a downward shift in CT_{max} value for stress-acclimated treatment. The present results clearly highlighted the detrimental combined effect of global climatic stressors and pollution on the physiological energetics of crabs that might potentially reduce their population and affect coastal aquaculture in forthcoming years.

Keywords Multiple stressors · Ocean acidification · Warming · Sundarbans · Oil spill · Crab · Scope for growth · CTmax

Introduction

In recent years, marine ecosystems are encountering numerous environmental challenges and myriad anthropogenic perturbations. It is now unequivocally acknowledged that the intense emission of atmospheric CO_2 is the prime cause of global climatic changes. Based on current trends, an escalation of 2–4°C average surface water temperature alongside a diminution by up to 0.4 pH units is predicted by the year 2100 under a business-as-usual scenario due to the unremitting rise of atmospheric greenhouse gas concentration (IPCC 2014, 2019). Hence, it is important to unravel the changes in the biological response under altered pH and temperature in contemporary natural variability as well as predicted future global changes (Hu et al. 2015). Temperature elevation is a crucial environmental factor regulating the physiological energetics of aquatic ectotherms (Huey and Kingsolver 1989; Hochachka and Somero 2002). Tropical invertebrates are more susceptible as they are the stenotherm with restricted thermal tolerance windows and reside close to their optimal thermal limit (Deutsch et al. 2008). Furthermore, it has been evinced that a rise in temperature also shifts animals' sensitivity toward other pollutants (Coppola et al. 2017; Andrade et al. 2019; Baag et al. 2021). Since the industrial revolution,

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The influence of ocean acidification and warming on responses of *Scylla serrata* to oil pollution: An integrated biomarker approach

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ABSTRACT

Anthropogenic activities primarily combustion of fossil fuel is the prime cause behind the increased concentration of CO_2 into the atmosphere. As a consequence, marine environments are anticipated to experience shift towards lower pH and elevated temperatures. Moreover, since the industrial revolution the growing demand for petroleum-based products has been mounting up worldwide leading to severe oil pollution. Sundarbans estuarine system (SES) is experiencing ocean warming, acidification as well as oil pollution from the last couple of decades. *Scylla serrata* is one of the most commercially significant species for aquaculture in coastal areas of Sundarbans. Thus, the prime objective of this study is to delineate whether exposure under ocean warming and acidification exacerbates effect of oil spill on oxidative stress of an estuarine crab *S. serrata*. Animals were separately exposed under current and projected climate change scenario for 30 days. After this half animals of each treatment were exposed to oil spill conditions for 24 h. Oxidative stress status superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), lipid peroxidation (LPO level) and DNA damage (Comet assay) were measured. Augmented antioxidant and detoxification enzyme activity was noted except for SOD but failed to counteract LPO and DNA damage. The present results clearly highlighted the detrimental combined effect of OWA and pollution on oxidative stress status of crabs that might potentially reduce its population and affect the coastal aquaculture in impending years.

1. Introduction

Environmental perturbations as a result of global climate change pose serious threats in marine ecosystems. Industrialization and human population growth worldwide are identified to be the major drivers of global climate change (Hashmi and Alam, 2019; Ghazali and Ali, 2019). Anthropogenic activities, primarily fossil fuel combustion, have increased the concentration of CO_2 in the atmosphere, altering the global carbon cycle and affecting climate patterns globally (Friedlingstein et al., 2019). The pH of marine waters is reducing (known colloquially as 'ocean acidification') and ocean temperatures are rising, with marine heat waves becoming more frequent (Caldeira and Wickett, 2003; Sokolov et al., 2009). Ocean acidification and warming are occurring concurrently, and the response of animals to projected conditions are likely the result of the cumulative effects of these and other key drivers such as habitat destruction, overfishing, pollution and contamination (Boyd et al., 2018; Britton et al., 2020). Moreover, since the industrial revolution the growing demand for petroleum-based products has been mounting up worldwide, leading to severe oil pollution in coastal ecosystems from various sources, majorly including oil spills and leakage from transportation.

Sundarbans, the largest mangrove forest in the world, is a UNESCO world heritage site and recently declared Ramsar site (Bhowmik and Mandal, 2021). However, the area is far from pristine, with enormous local and industrial waste discharges having been observed in the Sundarbans estuarine system (SES) (Zanardi-Lamardo et al., 2019). Additionally, the coastal Bay of Bengal including the SES has been experiencing steady surface water warming for the past couple of decades (Mitra et al., 2009; Samanta et al., 2018), and ocean pH has also altered due to the excessive absorption of CO_2 by the coastal waters in this area (Sarma et al., 2021). Fuel and oil spills from nearby Diamond Harbour and Haldia port are common (Panigrahy et al., 2014). Such spills introduce polycyclic aromatic hydrocarbons (PAHs) into coastal environments (Martins et al., 2011; Abreu-Mota et al., 2014). PAHs are presumed to affect aquatic biota in numerous ways, e.g., oxidative stress (Lushchak, 2011). Escalation in temperature also leads to overproduction and accumulation of reactive oxygen species (ROS) that cause oxidative damage to various cellular components (Lesser, 2006;

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Certificate of Participation

This is to certify that **Sritama Baag,** Department of Life Sciences, Presidency University, Kolkata has participated and presented a poster entitled *"Physiological Energetics of the Freshwater Gastropod Bellamya bengalensis Exposed to Thermal Stress"* in the **"6th India Biodiversity Meet 2019 (International Conference)"**, held at Indian Statistical Institute, Kolkata, from 14th - 16th February, 2019.

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7th National Conference of the Ocean Society of India (OSICON-21)

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Paper Title : The physiological response of Scylla serrata under the influence of climate change related factors and oil pollution

Anilkumar

Dr. Anilkumar N
Convenor, OSICON-21

M. Ravichandran

Dr. M. Ravichandran
President, OSI

Certificate of Participation

This is to certify that

Sritama Baag

Presidency University, Kolkata

presented ***POSTER*** in the two day **International Conference (Online) on**

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has participated in **ICBWOA** 2020 held at **IISER Kolkata** from 26th to 29th January, 2020

Punyasloke Bhadury
Convener, ICBWOA,
SwarnaJayanti Fellow,
Integrative Taxonomy and Microbial Ecology
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&
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Richard Bellerby
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Norwegian Institute for Water Research, Norway.
Distinguished Thousand Talents National Expert Professor
Director SKLEC-NIVA Centre for Marine and Coastal Research
State Key Laboratory for Estuarine and Coastal Research
East China Normal University, China

THE ZOOLOGICAL SOCIETY KOLKATA

SUDEV BHUSAN GHOSH EXCELLENCE AWARD, 2022

for Outstanding Presentation

is presented to

Ms. Sritama Baag

ON 23rd JULY,2022

PRESIDENT

HONY. GENERAL SECRETARY

THE ZOOLOGICAL SOCIETY KOLKATA

Participation Certificate for

R.K SUR MEMORIAL AWARD, 2022

is presented to

Ms. Sritama Baag

on 30th July 2022

President

Hony. general secretary